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The health assessment document for Polychlorinated dibenzofurans (PCDFs) provides the scientific data base for utilization by U.S. EPA in its activities related to the Clean Air Act. PCDFs are a group of widespread and environmentally stable halogenated tricyclic aromatic hydrocarbons. They enter the environment as trace impurities in PCBs, Chlorophenols, PCNs, 2,4,5-T formulations and as a result of diverse combustion processes. PCBFs bioaccumulate in exposed human and animal adipose rich tissues. They are extremely toxic to animals and humans. Their toxic effects are very similar to those caused by 2,3,7,8-TCDD. Information on adverse health effects in humans has been derived from the observation in the isolated Japanese and Tiawanese population who accidentally consumed PCBs and PCDF-contaminatted rice oil.

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This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

PREFACE

The Office of Health and Environmental Assessment has prepared this Health Assessment Document on polychlorinated dibenzofurans at the request of the Office of Air Quality Planning and Standards.

In the development of this assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary and conclusions have been prepared such that the toxicity of polychlorinated dibenzofurans is qualitatively and, where possible, quantitatively identified. Observed effect levels and dose-response relationships are discussed where appropriate in order to identify the critical effect and to place adverse health responses in perspective with observed environmental levels.

This document was reviewed by a panel of expert scientists during the peer review workshop held at U.S. EPA, Cincinnati, OH, on May 29 and 30, 1986.

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1. INTRODUCTION

Polychlorinated dibenzofurans (PCDFs) are members of a group of the widespread and environmentally stable halogenated tricyclic aromatic hydrocarbons. This group is structurally similar to polychlorinated biphenys (PCBs), polychlorinated napthalenes (PCNs), polychlorinated dibenzo-*p*dioxins (PCDDs), polybrominated biphenyls (PBBs) and polychlorinated biphenylenes. PCDFs are not deliberately disseminated in the environment as primary industrial products, but enter the environment as unintentional trace impurities in PCBs, chlorinated phenols, PCNs, 2,4,5-T formulations and as a result of diverse combustion processes. PCDFs have been detected in fly ash and in flue gas from municipal and industrial incinerators, and have been found to bioaccumulate in seal, fish, turtle and human adipose tissue. Residues of PCDFs have also been detected in human breast milk. As a result of PCDF detection in milk from Swedish mothers it has been suggested that primary PCDF exposure to this population is occurring from municipal incinerators. Consequently, in 1985 the National Swedish Environmental Protection Board decided on a moratorium for new municipal incinerators.

PCDFs are extremely toxic to animals and humans. Signs and symptoms of toxicity are very similar to those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Of the total 135 possible isomer congeners so far determined for PCDFs, 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF seem to be the most toxic. However, the relative toxicity of all the congeners have not yet been fully studied. The guinea pig is the most sensitive species to toxic effects of PCDF. Mice and rats are less sensitive than guinea pigs. Like PCDDs, these compounds are strong inducers of aryl hydrocarbon hydroxylase (AHH) and the most acutely toxic isomers are also the most potent inducers of AHH activity.

Mutagenicity data on PCDFs are extremely limited and three isomers studied gave negative responses in short-term microbial mutagenesis assay.

Recent observations indicate that 2,3,7,8-TCDF is teratogenic in mice resulting in dose-related increases in both isolated cleft palates and hydronephrosis in the fetus. Animal experimentation data indicate that PCDFs are capable of being transferred to the developing offspring, prenatally through transplacental migration and postnatally through the nursing mother's milk. When these two rates of transfer were compared, it was found that PCDF transported through nursing mother's milk appeared to be higher than transplacental migration to the fetus.

Information on adverse health effects in humans has been observed from the Japanese and Taiwanese population who consumed PCB- and PCDF-contaminated rice oil in 1968 and 1979, respectively. Severe adverse toxic effects, many of which are similar to those caused by 2,3,7,8-TCDD, were observed in the affected population.

Though PCDFs have been detected in a number of chemicals as contaminants and in the emissions from waste incinerators, a chronic toxicity data base for this class of compounds is not available. Primarily because of their prevalence in the environment and structural similarities between PCDFs and PCDDs, there is concern as to the public health impact of their presence in the environment.

2. PHYSICAL AND CHEMICAL PROPERTIES

2.1. SUMMARY

The dibenzofurans (DBFs) are a group of organic compounds that contain two benzene rings annulated to a central furan ring. The benzene rings can be substituted at the 1,2,3,4,6,7,8,9 positions. For a single substituent like chlorine there are 135 congeners over the substitution range mono- to octa-: these include 4 mono-, 16 di-, 28 tri-, 38 tetra-, 28 penta-, 16 hexa-, 4 hepta- and 1 octa-.

The crystal structure of the 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) is monoclinic. The latter is a structural analogue of the highly toxic 2,3,7,8-TCDD. The two benzene rings are coplanar except for the 1,2- and 1,9-substituted derivatives. Most polyhalogenated dibenzofurans (PHDFs), including the polychlorinated dibenzofurans (PCDFs), polyfluorinated dibenzofurans (PFDFs), polybrominated dibenzofurans (PBDFs) and the polyiodinated dibenzofurans (PIDFs) are solids at room temperature and have relatively high boiling points, which increase with degree of substitution. PHDFs are generally quite soluble in organic solvents but water solubility decreases with chlorination. The octanol/water partition coefficients of PCDFs increase with chlorine number; $\log K_{ow}$ values for 2,8-dichlorodibenzofuran (2,8-DCDF), 2,3,7,8-TCDF, and the octachlorodibenzofuran (OCDF) are 5.30, 5.82±0.02, and 8.78, respectively. Vapor pressure data for some PCDFs are available. The less polar the PHDF, the more readily it will adsorb to organic matter.

The PHDFs are aromatic compounds, and thus there are at least two distinct UV absorption maxima near 217 nm ($\sigma^* \leftarrow \sigma$ transition) and near 280 nm ($\pi^* \leftarrow \pi$ transition). The effect of ring substitution on absorption maxima tends to be minor. The proton and ^{13}C-nuclear magnetic resonance

(NMR) spectra are characteristic of aromatic molecules. As expected of aromatic molecules, the parent ions in the mass spectra are intense and are suitable for specific ion monitoring. The major fragment ions are M-Cl, M-Cl_2, M-Cl-CO, and M-Cl_2-CO. The major cleavage patterns are determined by the great stability of the ring system so that the fragmentations tend to be directed by the substituents.

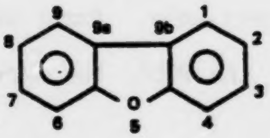
The PHDFs exhibit reactions directed by the substituents of the most substituted ring towards the least substituted ring.

Higher PCDFs can be degraded by ultraviolet (UV) light. These reactions are dependent on solvent and wavelength. Degradation is slow at 310 nm, but rapid at 254 nm or at 310 nm in the presence of suitable triplet sensitizers.

PHDF can also be produced by photodecomposition of higher PHDFs, by photocyclization or pyrolysis of halogenated diphenyl ethers, by pyrolysis of polyhalogenated biphenyls (PHBs) and halobenzenes, by halogenation of lower PHDFs, by palladium acetate cyclization of substituted diphenyl ethers, and by cyclization of substituted hydroxylated PHBs, and substituted diazotized halophenoxy-o-anilines.

2.2. INTRODUCTION

The accepted system of numbering of PHDFs is now as follows:



The ring that is most substituted has priority in numbering. For equally substituted rings, the numbering that allows the lowest sum of the substituted positions is the correct one.

There are 135 positional isomers for a single substituent: 4 mono-, 16 di-, 28 tri-, 38 tetra-, 28 penta-, 16 hexa-, 4 hepta- and 1 octa-. There has been little investigation of the physico-chemical properties of such compounds.

The Chemical Abstracts System Registry Numbers (CAS RN) for the PHDFs that have been cited in the literature are provided in Table 2-1.

2.3. PHYSICAL PROPERTIES

2.3.1. Molecular Structure. The acutely toxic 2,3,7,8-TCDF belongs to the monoclinic space group C2/c and the molecule is planar (Table 2-2). A crystallographic 2-fold axis bisects the bond between the rings and passes through the oxygen atom. The longest carbon-carbon bond distance within the benzenoid rings joins the carbon atoms to which the chlorine atoms are attached (Hubbard et al., 1978). Thus, the compound is very closely related in structure to the highly toxic 2,3,7,8-TCDD (Boer et al., 1972). No X-ray data for the other PHDFs are available.

The electronic structures of 25 PCDFs were characterized by Cheney and Tolly (1979). The powder X-ray diffraction patterns of 4 PCDFs have been reported by Cantrell et al. (1986). An unspecified MCDF, 3,6-DCDF, 2,3,7,8-TCDF, and OCDF were examined. The DCDF is easily distinguished from OCDF and the TCDF by its intense diffraction at 24.35 2θ, and the TCDF by its strong line at 15.20 2θ. The OCDF can with difficulty be detected by its diffraction at 31.81 2θ.

2.3.2. Melting Points. Table 2-3 gives the melting points of some PHDFs. Norstrom et al. (1979) showed by gas chromatography/mass spectrometry (GC/MS) that the PCDFs for which they determined melting points were pure. Kuroki et al. (1984) have published melting points for other PCDFs (Table 2-3).

TABLE 2-1

Chemical Abstracts System Registry Numbers (CAS-RN) for PHDFs

Chemical	Substitution	CAS-RN
PCDF		
Mono	mono	42934-53-2
	2-	84761-86-4
	3-	25074-67-3
	4-	74992-96-4
Di	di	43047-99-0
	1,2-	64126-85-8
	1,3-	94538-00-8
	1,4-	94538-01-9
	1,6-	74992-97-5
	1,7-	94538-02-0
	1,8-	81638-37-1
	1,9-	70648-14-5
	2,3-	64126-86-9
	2,4-	24478-74-8
	2,6-	60390-27-4
	2,7-	74992-98-6
	2,8-	5409-83-6
	3,4-	94570-83-9
	3,6-	74918-40-4
	3,7-	58802-21-4
	4,6-	64560-13-0
Tri	tri	43048-00-6
	1,2,3-	83636-47-9
	1,2,4-	24478-73-7
	1,2,6-	64560-15-2
	1,2,7-	83704-37-4
	1,2,8-	83704-34-1
	1,2,9-	83704-38-5
	1,3,4-	82911-61-3
	1,3,6-	83704-39-6
	1,3,7-	64560-16-3
	1,3,8-	76621-12-0
	1,3,9-	83704-40-9
	1,4,6-	82911-60-2
	1,4,7-	83704-41-0
	1,4,8-	64560-14-1
	1,4,9-	70648-13-4

TABLE 2-1 (cont.)

Chemical	Substitution	CAS-RN
PCDF (cont.)		
Tri (cont.)	2,3,4-	57117-34-7
	2,3,6-	57117-33-6
	2,3,7-	58802-17-8
	2,3,8-	57117-32-5
	2,3,9-	58802-18-9
	2,4,6-	58802-14-5
	2,4,7-	83704-42-1
	2,4,8-	54589-71-8
	2,4,9-	82911-59-9
	2,6,7-	83704-43-4
	3,4,6-	83704-43-2
	3,4,7-	83704-44-3
	3,4,8-	83704-45-4
	3,4,9-	83794-46-5
Tetra	tetra	30402-14-03
	1,2,3,4-	24478-72-6
	1,2,3,6-	83704-21-6
	1,2,3,7-	83704-22-7
	1,2,3,8-	62615-08-1
	1,2,3,9-	83704-23-8
	1,2,4,6-	71998-73-7
	1,2,4,7-	83719-40-8
	1,2,4,8-	64126-87-0
	1,2,4,9-	83704-24-9
	1,2,6,7-	83704-25-0
	1,2,6,8-	83710-07-0
	1,2,6,9-	70648-18-9
	1,2,7,8-	58802-20-3
	1,2,7,9-	83704-26-1
	1,2,8,9-	70648-22-5
	1,3,4,6-	83704-27-2
	1,3,4,7-	70648-16-7
	1,3,4,8-	64126-87-0
	1,3,4,9-	83704-28-3
	1,3,6,7-	57117-36-9
	1,3,6,8-	71998-72-6
	1,3,6,9-	83690-98-6
	1,3,7,8-	57117-35-8
	1,3,7,9-	64560-17-4
	1,4,6,7-	66794-59-0
	1,4,6,8-	82911-58-8
	1,4,6,9-	70648-19-0
	1,4,7,8-	83704-29-4

TABLE 2-1 (cont.)

Chemical	Substitution	CAS-RN
PCDF (cont.)		
Tetra (cont.)	2,3,4,6-	83704-30-7
	2,3,4,7-	83704-31-8
	2,3,4,8-	83704-32-9
	2,3,4,9-	83704-33-0
	2,3,6,7-	57117-39-2
	2,3,6,8-	57117-37-0
	2,3,7,8-	51207-31-9
	2,4,6,7-	57117-38-1
	2,4,6,8-	58802-19-0
	3,4,6,7-	57117-40-5
Penta	penta	30402-15-4
	1,2,3,4,6-	83704-47-6
	1,2,3,4,7-	83704-48-7
	1,2,3,4,8-	67517-48-0
	1,2,3,4,9-	83704-49-8
	1,2,3,6,7-	57117-42-7
	1,2,3,6,8-	83704-51-2
	1,2,3,6,9-	83704-52-3
	1,2,3,7,8-	57117-41-6
	1,2,3,7,9-	83704-53-4
	1,2,3,8,9-	83704-54-5
	1,2,3,6,7-	83704-50-1
	1,2,4,6,8-	69698-57-3
	1,2,4,6,9-	70648-24-7
	1,2,4,7,8-	58802-15-6
	1,2,4,7,9-	71998-74-8
	1,2,4,8,9-	70648-23-6
	1,2,6,7,8-	69433-00-7
	1,3,4,6,7-	83704-36-3
	1,3,4,6,8-	83704-55-6
	1,3,4,6,9-	70648-15-6
	1,3,4,7,8-	58802-16-7
	1,3,4,7,9-	70648-20-3
	1,3,4,8,9-	70872-82-1
	2,3,4,6,7-	57117-43-8
	2,3,4,6,8-	67481-22-5
	2,3,4,6,9-	83704-35-2
	2,3,4,7,8-	57117-31-4
	2,3,4,7,9-	70648-21-4

TABLE 2-1 (cont.)

Chemical	Substitution	CAS-RN
PCDF (cont.)		
Hexa	hexa	55684-94-1
	1,2,3,4,6,7-	79060-60-9
	1,2,3,4,6,8-	69698-60-8
	1,2,3,4,6,9-	91538-83-9
	1,2,3,4,7,8-	70648-26-9
	1,2,3,4,7,9-	91538-84-0
	1,2,3,4,8,9-	92341-07-6
	1,2,3,6,7,8-	57117-44-9
	1,2,3,6,7,9-	92341-06-5
	1,2,3,6,8,9-	75198-38-8
	1,2,3,7,8,9-	72918-21-9
	1,2,4,6,7,8-	67562-40-7
	1,2,4,6,7,9-	75627-02-0
	1,2,4,6,8,9-	69698-59-5
	1,3,4,6,7,8-	71998-75-9
	1,3,4,6,7,9-	92341-05-4
	2,3,4,6,7,8-	60851-34-5
Hepta	hepta	38998-75-3
	1,2,3,4,6,7,8-	67562-39-4
	1,2,3,4,6,7,9-	70648-25-8
	1,2,3,4,6,8,9-	69698-58-4
	1,2,3,4,7,8,9-	55673-89-7
Octa	octa	3268-87-9
	1,2,3,4,6,7,8,9-	39001-02-0
^{14}C	1,2,7,8-	78813-10-2
	2,3,7,8-	78813-09-9
	2,3,4,7,8-	87665-66-5
PBDF		
Mono	1-	50548-45-3
	2-	86-76-0
	3-	26608-06-0
	4-	89827-45-2
Di	2,7-	65489-80-7
	2,8-	10016-52-1
	3,7-	67019-91-4

TABLE 2-1 (cont.)

Chemical	Substitution	CAS-RN
PBDF (cont.)		
Tri		
	1,2,8-	84761-81-9
	2,3,8-	84761-82-0
Tetra		
	1,2,7,8-	84761-80-8
	2,3,7,8-	67733-57-7
Penta	penta	68795-14-2
Hepta	hepta	62994-32-5
PFDF		
Mono	2-	391-46-8
	3-	391-54-8
Di	2,7-	71277-77-5
	2,8-	10016-52-1
Tri	1,2,4-	15950-24-0
Tetra	1,2,3,4-	15945-39-8
Octa	octa	16804-47-0
PIDF		
Mono	2-	5408-56-0
	3-	5896-29-7
	4-	65344-26-5
Di	2,7-	81050-43-3
	2,8-	5943-11-3
	3,7-	5914-49-8
Mixed halo	tribromo-2,4,8-trichloro	54605-27-5
	tetrabromo-2,4,8-trichloro	54605-26-4

TABLE 2-2

X-Ray Crystallographic Indices of Some Selected PHDFs*

Dibenzofuran	Unit Cell Indices (Å)			Density (g/m/ℓ)		Bond Length (Å)
	a	b	c	(exptl)	(calc'd)	
2,3,7,8-TCDF	14.702(4)	12.886(4)	6.256(1)	1.72	1.74	(2,8)C-Cl(1):1.725(2) (3,7)C-Cl(2):1.732(2) C-O:1.385(2) Benz C-C:1.366(2) to 1.404(2)

*Source: Hubbard et al., 1978

TABLE 2-3

Melting and Boiling Points of Some PHDFs

Dibenzofuran	Melting Point (°C)	Boiling Point (°C)	Reference
--, 1-MBDF	67		Coffey, 1973
--, 2-MBDF	110		Coffey, 1973
	110	220 (40 mm Hg)	Weast, 1985
--, 3-MBDF	120		Coffey, 1973
	120	220 (40 mm Hg)	Weast, 1985
--, 4-MBDF	72		Coffey, 1973
	67		Weast, 1985
--, 2-MCDF	106		Coffey, 1973
--, 3-MCDF	101		Coffey, 1973
	101-102		Kuroki et al., 1984
--, 2-MIDF	112		Coffey, 1973
--, 3-MIDF	142		Coffey, 1973
--, 2-MFDF	89		Coffey, 1973
--, 3-MFDF	88.5		Coffey, 1973
--, 2,8-DBDF	195		Coffey, 1973
	199-200		Weast, 1985
	191.5-192.5		Oshima et al., 1968c
--, 2,8-DCDF	185		Gilman and Young, 1934
	184-185		Kuroki et al., 1984
--, 3,7-DCDF	186-188		Norstrom et al., 1979
--, 2,8-DIDF	77		Coffey, 1973
--, 2,3,8-TrCDF	180-181		Norstrom et al., 1979
	189-191		Gray et al., 1976

TABLE 2-3 (cont.)

Dibenzofuran	Melting Point (°C)	Boiling Point (°C)	Reference
--, 2,4,6-TrCDF	116-117		Gray et al., 1976
--,2,4,8--TrCDF	155-156		Kuroki et al., 1984
--, 1,2,4--TrCDF	100-101		Coffey, 1973
--,1,3,6,8-TCDF	177-178		Kuroki et al., 1984
--,1,3,6,7-TCDF	176.5-177		Kuroki et al., 1984
--,1,3,7,9-TCDF	206.5-207.5		Kuroki et al., 1984
--,1,4,6,7-TCDF	180-181		Kuroki et al., 1984
--,2,3,6,8-TCDF	197-198 202-203		Kuroki et al., 1984 Gray et al., 1976
--,2,4,6,7-TCDF	164-164.5		Kuroki et al., 1984
--,1,2,7,8-TCDF	210-211		Kuroki et al., 1984
--,1,2,6,7-TCDF	199-200		Kuroki et al., 1984
--1,2,7,9-TCDF	184-185		Kuroki et al., 1984
--,2,3,7,8-TCDF	219-221 226-228 227-228		Kuroki et al., 1984 Norstrom et al., 1979 Gray et al., 1976
--,2,3,6,7-TCDF	195-196		Kuroki et al., 1984
--,2,4,6,8-TCDF	192-192.5 198-200		Kuroki et al., 1984 Gray et al., 1976

TABLE 2-3 (cont.)

Dibenzofuran	Melting Point (°C)	Boiling Point (°C)	Reference
--,1,2,3,7-TCDF	167.5-168		Kuroki et al., 1984
--,1,2,3,8-TCDF	197-198		Kuroki et al., 1984
--,2,3,4,8-TCDF	176-177		Kuroki et al., 1984
--,2,3,4,7-TCDF	172.5-173		Kuroki et al., 1984
--,2,3,4,6-TCDF	153-154		Kuroki et al., 1984
--,1,2,4,8-TCDF	191-193		Kuroki et al., 1984
--1,2,3,4-TCDF	168.5-169 169-170		Kuroki et al., 1984 Coffey, 1973
--,1,2,4,6,8-PeCDF	204-205		Kuroki et al., 1984
--,1,2,4,7,8-PeCDF	236-238 225.5-226.5		Kuroki et al., 1984 Norstrom et al., 1979
	234-235		Gray et al., 1976
--,1,2,4,7,9-PeCDF	196-197		Kuroki et al., 1984
--,1,2,3,7,8-PeCDF	225-227		Kuroki et al., 1984
--,1,2,3,6,7-PeCDF	205-207		Kuroki et al., 1984
--,1,2,6,7,8-PeCDF	220-221		Kuroki et al., 1984
--,2,3,4,7,8-PeCDF	196-196.5		Kuroki et al., 1984
--,2,3,4,6,7-PeCDF	201.5-202		Kuroki et al., 1984

TABLE 2-3 (cont.)

Dibenzofuran	Melting Point (°C)	Boiling Point (°C)	Reference
--,2,3,4,6,8-PeCDF	219-220		Kuroki et al., 1984
--,1,3,4,7,8-PeCDF	168-170		Kuroki et al., 1984
--,1,3,4,6,7-PeCDF	195-195.5		Kuroki et al., 1984
--,1,2,3,4,6-PeCDF	194-195		Kuroki et al., 1984
--,1,2,4,6,7-PeCDF	178.5-179.5		Kuroki et al., 1984
--,1,2,3,4,8-PeCDF	177-178		Kuroki et al., 1984
--,1,2,4,6,7,8-HxCDF	221-222		Kuroki et al., 1984
--,1,3,4,6,7,8-HxCDF	229-230		Kuroki et al., 1984
--,1,2,4,6,8,9-HxCDF	247-249		Norstrom et al., 1979
	246-248		Kuroki et al., 1984
--,1,2,3,4,6,7-HxCDF	227-228		Kuroki et al., 1984
--,1,2,3,4,7,8-HxCDF	225.5-226.5		Kuroki et al., 1984
--,1,2,3,6,7,8-HxCDF	232-234		Kuroki et al., 1984
--,1,2,3,4,6,7,8-HxCDF	239-240		Kuroki et al., 1984
--,1,2,3,6,8,9-HxCDF	206-207		Kuroki et al., 1984
--,1,2,4,6,7,9-HxCDF	180-181		Kuroki et al., 1984

TABLE 2-3 (cont.)

Dibenzofuran	Melting Point (°C)	Boiling Point (°C)	Reference
--,1,2,3,4,6,8-HxCDF	233.5-234		Kuroki et al., 1984
--,1,2,3,4,6,9-HxCDF	196-197		Kuroki et al., 1984
--,1,2,3,4,7,9-HxCDF	216-217		Kuroki et al., 1984
--,1,2,3,4,6,7,8-HpCDF	236-237		Kuroki et al., 1984
--,1,2,3,4,6,8,9-HpCDF	211-212		Kuroki et al., 1984
--,1,2,3,4,7,8,9-HpCDF	221-223		Kuroki et al., 1984
--, OFDF	100		Coffey, 1973

2.3.3. Solubility. PHDFs are less polar than dibenzofuran and would be expected to be less soluble in water and more soluble in organic solvents. Research needs to be done in this area. No solubility data even for the PCDFs have been reported. However, 2,3,7,8-TCDF is said to be slightly more soluble in water than is the corresponding dioxin, 2,3,7,8-TCDD (Greenlee and Poland, 1978), which has a solubility of ~0.2 µg/ℓ at 25°C (Helling et al., 1973).

2.3.4. Partition Coefficients. Sarna et al. (1984) and Burkhard and Kuehl (1986) have documented the octanol/water partition coefficients for some PCDFs (Table 2-4). The disagreement for OCDF arises because of uncertainties in the K_{ow} values of reference compounds of high K_{ow}. The partitioning of organic chemicals between lipid and water is an important determinant of the bioconcentration potential of a toxicant and has sometimes been effectively used as an indicator of the preferred degradative *in vivo* pathways.

2.3.5. Boiling Points and Vapor Pressures. No vapor pressures of any of the PHDFs at 25°C have been measured directly.

The vapor pressures for a series of PCDFs have been estimated by a gas-chromatographic method (Firestone, 1977a; Berg et al., 1985a) (Table 2-5). Unfortunately, different investigators have obtained different pressures by this method for the esters of 2,4-dichlorophenoxyacetic acid (Jensen and Schall, 1966; Flint et al., 1968). The vapor pressures, however, of the PCDFs will certainly not be lower than the values in Table 2-5, and a decrease in vapor pressure with increasing chlorination is expected.

2.3.6. Adsorption and Desorption. It would be expected that the PHDFs would adsorb very strongly to organic matter and nonpolar nonorganic material such as charcoal, but not so strongly to polar inorganic material

TABLE 2-4

The Logarithm of the Octanol/Water Partition Coefficients (K_{ow}) of Some PHDFs Using HPLC Methods

PCDF	log K_{ow}	Reference
2,8-dichloro-	5.95	Sarna et al., 1984[a]
	5.30[b]	Burkhard and Kuehl, 1986[c]
2,3,7,8-tetrachloro-	5.82±0.02	Burkhard and Kuehl, 1986[c]
octachloro-	13.37	Sarna et al., 1984[a]
	8.78	Burkhard and Kuehl, 1986[c]

[a] Quadratic equation treatment: Biorad Biosil (10 μm) data

[b] Quadratic equation treatment: Unspecified "microbore" HPLC column

[c] Sarna et al. (1984) data recalculated from experimental data

TABLE 2-5

Vapor Densities and Pressures of PCDFs as Estimated from a Gas Chromatographic Method*

PCDF Isomer	Estimated Vapor Pressure (mm Hg x 10^{-6} at 25°C)	Estimated Vapor Density at 25°C (μg/m³)
Di-		
2,4-	7.3	82
3,7-	7.0	79
2,8-	6.8	77
Tri-		
2,4,6-	4.0	52
2,3,8-	3.7	48
Tetra-		
1,4,6,8-	2.5	38
2,4,6,8-	2.5	38
2,3,4,8-	2.2	33
2,4,6,7-	2.1	32
1,2,7,8-	2.0	30
2,3,7,8-	2.0	30
2,3,6,7-	1.9	29
3,4,6,7-	1.8	27
Penta-		
1,3,4,7,8-	1.3	22
1,2,4,7,8-	1.3	22
1,2,3,6,7-	1.1	18
2,3,4,7,8-	1.1	18
Hepta-		
unspecified	0.44	9.0
unspecified	0.36	7.4
unspecified	0.30	6.2
Octa-	0.19	4.3

*Source: Firestone, 1977a

such as alumina. The predicted behavior is borne out by the fact that alumina is used generally for the column chromatography of PCDDs and PCDFs (O'Keefe, 1978a). Strong binding is expected to soils of high organic content as observed for 2,3,7,8-TCDD (Isensee, 1978). This has been observed for 2,3,7,8-TCDD in South Vietnam and northwestern Florida soils (Westing, 1978). Although both 2,3,7,8-TCDD and -TCDF are comparatively insoluble in water, the 2,3,7,8-TCDF appears to be slightly more water soluble. There is a possibility that the TCDF will be more readily leached from soils than TCDD. Experiments should be performed to test this hypothesis. The relation, Equation 2-1, has been shown to hold by Karickhoff et al. (1979) for nonpolar compounds.

$$\log K_{oc} = \log K_{ow} - 0.21 \tag{2-1}$$

where

K_{oc} is a constant that is characteristic of the compound under consideration and

K_{ow} is the octanol/water coefficient

If the K_{ow} values of Burkhard and Kuehl (1986) (see Table 2-4) are assumed, the log K_{oc} values for 2,8-DCDF, 2,3,7,8-TCDF, and OCDF are 5.09, 5.61 and 8.57, respectively.

If the PHDFs are adsorbed in the same manner as dibenzothiophene by soils (Hassett et al., 1980), the Freundlich adsorption isotherm should apply as follows:

$$C_s = K_d C_w^{1/n} \tag{2-2}$$

where

C_s is the amount sorbed in μg/g of soil

C_w is the equilibrium solution concentration (μg/ℓ)

n is an integer characteristic of the process and

K_d is the Freundlich constant

If n = 1, as for dibenzothiophene (Hassett et al., 1980),

$$C_S = K_d C_W \qquad (2\text{-}3)$$

Since

$$K_d = 100K_{oc}/\%OC \qquad (2\text{-}4)$$

where %OC is the percentage of soil organic content. For an organic content of 1%, $K_d = 100\ K_{oc}$. Thus, log K_d for 2,8-DCBF, 2,3,7,8-TCDF, and OCDF are then 7.09, 7.61 and 10.57, respectively.

2.3.7. Miscellaneous Properties. The molar refractions of the DCDFs, TrCDFs, TCDFs, PeCDFs, HpCDFs and OCDF have been estimated to be 60.2, 65.0, 69.8, 74.6, 84.2 and 89.0, respectively (Firestone, 1977a).

2.3.8. Spectroscopic Properties.

2.3.8.1. ULTRAVIOLET ABSORPTION SPECTRA -- There are few studies of the UV absorption spectra of PHDFs. The existing data for MBDFs are provided by Weast (1985) in Table 2-6 and for PCDFs by Firestone (1977a) and Gray et al., (1976) in Table 2-7.

The UV spectra of some 2-MHDFs have been reported by Kuroki (1968). The Hammett ρ value for the 2-substituted derivatives was 1.44. Thus, there was not much effect on λ_{max} of substitution at this position (Kuroki, 1968).

Chloro-substitution does not affect the λ_{max} of the parent compound very much for the 2-MCDF and 3-MCDF (Cerniani et al., 1954). The UV spectrum of 2,8-DCDF is also similar to that of unsubstituted DF with a λ_{max} at 290 nm in methanol (Crosby et al., 1973). Firestone (1977a) reported the UV absorptions of several PCDFs and found the red shift on increasing chlorination was small as shown in Table 2-7.

2.3.8.2. FLUORESCENCE -- No fluorescence data for the PHDFs have yet been published.

TABLE 2-6

Ultraviolet Absorption Maxima and Molar Absorptivities for Some Substituted PBDFs*

MBDF	Solvent	Absorption Peak in nm [log(molar absorptivity)]
2-Bromo	Acetic acid	222(4.58), 251(4.19), 288(4.25), 310(3.67)
3-Bromo	Ethanol	219(4.55), 254(4.31), 290(4.31), 300(4.22)

*Source: Weast, 1985

TABLE 2-7

Absorption Maxima of Some PCDFs in Chloroform[a]

PCDF	UV Absorption Maxima Wavelengths (nm)
2,3,8-TrCDF	256, 302, 313
2,3,6,8-TCDF	250, 260, 285, 297, 314[b]
2,3,7,8-TCDF	257, 294, 310, 323
2,4,6,8-TCDF	259, 309, 316
1,2,4,7,8-PeCDF	256, 266, 297
1,3,4,7,8-PeCDF	263, 272, 297, 320

[a] Source: Firestone, 1977a

[b] Gray et al., 1976

2.3.8.3. PHOSPHORESCENCE -- Few studies have been reported on the phosphorescence of the PHDFs. Teplyakov et al. (1970) noted an increase in quantum efficiency on substitution of dibenzofuran with chlorine atoms. The use of phosphorescence techniques to determine PCDFs has been suggested by Brownrigg et al. (1972). The difficulties of low-temperature technology and the interference by impurities are substantial.

2.3.8.4. NMR AND EPR SPECTROSCOPY --The proton and ^{19}F spectra of 1,2,3,4-TFDF and 1,2,4-TrFDF implied that there was a large para F-F coupling (Brown and Mooney, 1967). The proton NMR data of some PCDFs are given in Table 2-8.

The ^{13}C-NMR chemical shifts and assignments of carbon-proton coupling constants have been reported for the 2-MBDF and 2,8-DBDF. Long range substituent effects were observed in unsymmetrical compounds (Huckerby, 1979).

2.3.8.5. MASS SPECTROSCOPY -- The spectra for the 2,3,7,8-TCDF and OCDF (Curley et al., 1974) attest to the great stability of the parent ion since the latter is the largest peak in the spectrum.

The mass spectra of the PHDFs will be discussed more thoroughly in the section on GC/MS (Section 3.4.2.2.2). The major fragment ions are M-Cl, $M-Cl_2$, M-Cl-CO and $M-Cl_2-CO$.

2.4. CHEMICAL REACTIONS

2.4.1. General. PHDF reactions have been reviewed by Coffey (1973); Gilman's group was largely responsible for much of the early chemistry (Gilman et al., 1934a,b, 1935a,b, 1939a,b,c,d, 1940a,b,c, 1944; Gilman and Young, 1934, 1935; Gilman and Ess, 1939; Gilman and Abbott, 1943; Gilman and Thirtle, 1944; Gilman and Swiss, 1944; Gilman and Avakian, 1945a,b; Gilman and Esmay, 1953, 1954).

TABLE 2-8

Proton NMR Data for Some PCDFs

PCDF	Chemical Shifts (ppm downfield from tetramethylsilane)	Coupling Constants (J) (Hertz)	Reference
1-	m, 7.20-8.10		Safe and Safe, 1984
2-	m, 7.20-8.10		Safe and Safe, 1984
3-	m, 7.20-8.10		Safe and Safe, 1984
4-	m, 7.20-8.10		Safe and Safe, 1984
2,3-di-	m, 7.20-8.10		Safe and Safe, 1984
2,6-di-	m, 7.20-8.20		Safe and Safe, 1984
2,7-di-	m, 7.20-8.20		Safe and Safe, 1984
2,8-di-	m, 7.20-8.10 m, $H_{3,4,6,7} = 7.45-7.47$; $H_{1,9} = 7.87$	 $J_{1,9} = 1.1; 1.8$	Safe and Safe, 1984 Kuroki et al., 1984
3,7-di-	$H_1 = 7.98$; $H_2 = 7.38$; $H_4 = 7.68$	$J_{1,2} = 8.30$; $J_{2,4} = 1.40$	Norstrom et al., 1979
1,3,6-tri-	$H_2 = 7.55$; $H_4 = 7.85$; $H_7 = 7.68$; $H_8 = 7.50$; $H_9 = 8.27$	$J_{2,4} = 1.6$; $J_{7,8} = 8.1$; $J_{8,9} = 8.1$; $J_{7,9} = 1.2$	Safe and Safe, 1984
1,3,8-tri-	$H_2 = 7.55$; $H_4 = 7.79$; $H_6 = 7.76$; $H_7 = 7.64$; $H_9 = 8.27$	$J_{2,4} = 1.6$; $J_{6,7} = 8.4$; $J_{7,9} = 2.2$	Safe and Safe, 1984
2,3,4-tri-	m, H_6 to $H_9 = 7.25-8.25$; $H_1 = 8.39$		Safe and Safe, 1984
2,3,8-tri-	$H_1 = 8.27$; $H_4 = 7.82$; $H_6 = 7.56$; $H_7 = 7.45$; $H_9 = 8.09$ $H_1 = 8.07$; $H_{6,7} = 7.54$ (m); $H_9 = 8.24$	$J_{6,7} = 8.6$; $J_{7,9} = 2.2$	Safe and Safe, 1984 Norstrom et al., 1979
2,4,8-tri-	$H_1 = 7.77$; $H_3 = 7.49$; $H_6 = 7.56$; $H_7 = 7.48$; $H_9 = 7.87$	$J_{1,3} = 2.0$; $J_{6,7} = 8.8$; $J_{7.9} = 1.8$	Kuroki et al., 1984
2,6,7-tri-	$H_1 = 8.08$; $H_3 = 7.46$; $H_4 = 7.63$; $H_8 = 7.48$; $H_9 = 7.99$	$J_{1,3} = 2.4$; $H_{3,4} = 8.2$; $J_{8.9} = 8.4$	Safe and Safe, 1984
1,2,3,4-tetra-	$H_6 = 7.65$; $H_7 = 7.58$; $H_8 = 7.44$; $H_9 = 8.34$	$J_{7.8; 6,7; 7,8} = 7.0$; $J_{6,8/7,9} = 2.0$	Bell and Gara, 1985
	m, 8.29-8.40 for H_9 m, 7.33-7.76 for $H_{6,7,8}$		Kuroki et al., 1984
1,2,3,7-tetra-	$H_4 = 7.63$; $H_6 = 7.57$; H_8 7.38; $H_9 = 8.23$ $H_4 = 7.65$; $H_6 = 7.60$; $H_8 = 7.41$; $H_9 = 8.25$	$J_{8.9} = 8.4$; $H_{6,8} = 1.8$	Kuroki et al., 1984

TABLE 2-8 (cont.)

PCDF	Chemical Shifts (ppm downfield from tetramethylsilane)	Coupling Constants (J) (Hertz)	Reference
1,2,3,8-tetra-	$H_4 = 7.64$; $H_{6,7} = 7.50$; $H_9 = 8.29$	$J_{7,9} = 1.3$	Kuroki et al., 1984
1,2,4,7-tetra-	$H_3 = 7.59$; $H_6 = 7.67$; $H_8 = 7.43$; $H_9 = 8.28$	$J_{8,9} = 8.3$; $J_{6,8} = 1.8$	Bell and Gara, 1985
1,2,4,8-tetra-	$H_3 = 7.60$; $H_{6,7} = m, 7.45\text{-}7.56$; $H_9 = 8.31$ $H_3 = 7.76$; $H_6 = 7.71$; $H_7 = 7.58$; $H_9 = 8.25$	$J_{7,9} = 1.1$ $J_{6,7} = 9.0$; $J_{7,9} = 2.3$	Kuroki et al., 1984 Safe and Safe, 1984
1,2,6,7-tetra-	$H_3 = 7.50$; $H_4 = 7.57$; $H_8 = 7.50$; $H_9 = 8.17$ $H_3 = 7.52$; $H_4 = 8.20$; $H_8 = 7.62$; $H_9 = 7.49$	$J_{3,4} = 8.8$; $J_{8,9} = 8.5$ $J_{3,4} = 8.7$; $J_{8,9} = 8.7$	Kuroki et al., 1984 Bell and Gara, 1985
1,2,7,8-tetra-	$H_3 = 7.43$; $H_4 = 7.57$; $H_6 = 7.71$; $H_9 = 8.44$	$J_{3,4} = 8.8$; $J_{6,9} = 0.4$	Kuroki et al., 1984
1,2,7,9-tetra-	$H_3 = 7.43$; $H_4 = 7.61$; $H_6 = 7.49$; $H_8 = 7.43$	$J_{3,4} = 8.8$; $J_{6,8} = 1.9$	Kuroki et al., 1984
1,3,4,7-tetra-	$H_2 = 7.45$; $H_6 = 7.64$; $H_8 = 7.40$; $H_9 = 8.19$	$J_{8,9} = 8.9$; $J_{6,8} = 2.0$	Bell and Gara, 1985
1,3,6,7-tetra-	$H_2 = 7.36$; $H_4 = 7.54$; $H_8 = 7.46$; $H_9 = 8.06$	$J_{8,9} = 8.5$; $J_{2,4} = 1.65$	Kuroki et al., 1984
1,3,6,8-tetra-	$H_2 = 7.39$; $H_4 = 7.56$; $H_7 = 7.52$; $H_9 = 8.16$ $H_2 = 7.39$; $H_4 = 7.56$; $H_7 = 7.52$; $H_9 = 8.16$	$J_{2,4} = 1.5$; $J_{7,9} = 1.5$ $J_{2,4} = 1.8$; $J_{7,9} = 2.0$	Kuroki et al., 1984 Bell and Gara, 1985
1,3,7,9-tetra-	$H_{2,8} = 7.41$; $H_{4,6} = 7.48$	$J_{2,4} = 2.0$; $J_{6,8} = 2.0$	Kuroki et al., 1984
1,4,6,7-tetra-	$H_2 = 7.30$; $H_3 = 7.43$; $H_8 = 7.51$; $H_9 = 8.14$	$J_{2,3} = 8.4$; $J_{8,9} = 8.5$	Kuroki et al., 1984
2,3,4,6-tetra-	$H_1 = 7.95$; $H_7 = 7.55$; $H_8 = 7.33$; $H_9 = 7.79$ $H_1 = 8.39$; $H_7 = 7.69$; $H_8 = 7.51$; $H_9 = 8.18$	$J_{8,9} = 7.4$; $J_{7,8} = 7.6$ $J_{7,9} = 1.5$ $J_{8,9} = 7.8$; $J_{7,8} = 7.8$ $J_{7,9} = 1.2$	Kuroki et al., 1984 Safe and Safe, 1984
2,3,4,7-tetra-	$H_1 = 7.91$; $H_6 = 7.64$; $H_8 = 7.39$; $H_9 = 7.79$ $H_1 = 7.88$; $H_6 = 7.62$; $H_8 = 7.36$; $H_9 = 7.77$	$J_{8,9} = 8.3$; $J_{6,8} = 1.6$ $J_{8,9} = 7.9$; $J_{6,8} = 2.0$	Kuroki et al., 1984 Bell and Gara, 1985
2,3,4,8-tetra-	$H_1 = 7.92$; $H_6 = 7.57$; $H_7 = 7.50$; $H_9 = 7.87$ $H_1 = 7.87$; $H_6 = 7.54$; $H_7 = 7.46$; $H_9 = 7.82$ $H_1 = 8.39$; $H_6 = 7.78$; $H_7 = 7.64$; $H_9 = 8.18$	$J_{6,7} = 8.8$; $J_{7,9} = 1.8$ $J_{6,7} = 8.8$; $J_{7,9} = 2.0$ $J_{6,7} = 8.8$; $J_{7,9} = 2.2$	Kuroki et al., 1984 Bell and Gara, 1985 Safe and Safe, 1984
2,3,6,7-tetra-	$H_1 = 7.99$; $H_4 = 7.77$; $H_8 = 7.47$; $H_9 = 7.71$	$J_{8,9} = 8.5$	Kuroki et al., 1984
2,3,6,8-tetra-	$H_1 = 7.98$; $H_4 = 7.76$; $H_7 = 7.51$; $H_9 = 7.77$ $H_1 = 8.44$; $H_4 = 8.06$; $H_7 = 7.70$; $H_9 = 8.23$	$J_{7,9} = 1.65$ $J_{7,9} = 2.0$	Kuroki et al., 1984 Bell and Gara, 1985
2,3,7,8-tetra-	$H_{1,9} = 7.90$; $H_{4,6} = 7.70$ $H_{1,9} = 7.94$; $H_{4,6} = 7.67$ $H_{1,9} = 7.89$; $H_{4,6} = ?$		Kuroki et al., 1984 Bell and Gara, 1985 Norstrom et al., 1979

TABLE 2-8 (cont.)

PCDF	Chemical Shifts (ppm downfield from tetramethylsilane)	Coupling Constants (J) (Hertz)	Reference
2,4,6-7-tetra-	$H_1 = 7.79$; $H_3 = 7.51$; $H_8 = 7.48$; $H_9 = 7.72$ $H_1 = 8.23$; $H_3 = 7.73$; $H_8 = 7.70$; $H_9 = 8.18$	$J_{8,9} = 8.45$; $J_{1,3} = 2.1$ $J_{8,9} = 8.3$; $J_{1,3} = 1.9$	Kuroki et al., 1984 Bell and Gara, 1985
2,4,6,8-tetra-	$H_{1,9} = 7.77$; $H_{3,7} = 7.53$	$J_{1,3;\ 7,9} = 1.8$	Kuroki et al., 1984
3,4,6,7-tetra-	$H_{1,9} = 7.70$; $H_{2,8} = 7.46$	$J_{8,9;\ 1,2} = 8.7$	Bell and Gara, 1985
1,2,3,4,6-penta-	$H_7 = 7.58$; $H_8 = 7.38$; $H_4 = 8.24$	$J_{7,8} = 7.75$; $J_{8.9} = 7.55$; $J_{7.9} = 1.4$	Kuroki et al., 1984
1,2,3,4,8-penta-	$H_{6,7} = m$; 7.55-7.56; $H_9 = 8.27$ $H_6 = 7.89$; $H_7 = 7.74$; $H_9 = 8.37$	$J_{7.9} = 1.3$ $J_{6,7} = 8.6$; $J_{7,9} = 2.2$	Kuroki et al., 1984 Safe and Safe, 1984
1,2,3,6,7-penta-	$H_4 = 7.72$; $H_8 = 7.52$; $H_9 = 8.15$ $H_4 = 7.71$; $H_8 = 7.45$; $H_9 = 8.13$	$J_{8,9} = 8.6$ $J_{8,9} = 8.5$	Kuroki et al., 1984 Bell and Gara, 1985
1,2,3,6,9-penta-	$H_4 = 7.71$; $H_7 = 7.43$; $H_8 = 7.34$	$J_{7,8} = 8.5$	Bell and Gara, 1985
1,2,3,7,8-penta-	$H_4 = 7.65$; $H_6 = 7.71$; $H_9 = 8.40$ $H_4 = 8.06$; $H_6 = 8.08$; $H_9 = 8.52$ $H_4 = 7.69$; $H_6 = 7.64$; $H_9 = 8.39$		Kuroki et al., 1984 Safe and Safe, 1984 Bell and Gara, 1985
1,2,3,8,9-penta-	$H_4 = 7.65$; $H_6 = 7.64$; $H_7 = 7.43$	$J_{6,7} = 8.6$	Bell and Gara, 1985
1,2,4,6,7-penta-	$H_3 = 7.63$; $H_8 = 7.54$; $H_9 = 8.19$ $H_3 = 7.60$; $H_8 = 7.51$; $H_9 = 8.15$	$J_{8,9} = 8.7$ $J_{8,9} = 8.6$	Kuroki et al., 1984 Bell and Gara, 1985
1,2,4,6,8-penta-	$H_3 = 7.65$; $H_7 = 7.59$; $H_9 = 8.25$	$J_{7,9} = 2.1$	Kuroki et al., 1984
1,2,4,6,9-penta-	$H_3 = 7.69$; $H_7 = 7.46$; $H_8 = 7.37$	$J_{7,8} = 8.5$	Bell and Gara, 1985
1,2,4,7,8-penta-	$H_3 = 7.63$; $H_6 = 7.79$; $H_9 = 8.45$		Kuroki et al., 1984
1,2,4,7,9-penta-	$H_3 = 7.68$; $H_6 = 7.58$; $H_8 = 7.47$	$J_{6,8} = 2.0$	Kuroki et al., 1984
1,2,4,8,9-penta-	$H_3 = 7.69$; $H_6 = 7.67$; $H_7 = 7.52$	$J_{6,7} = 8.8$	Bell and Gara, 1985
1,2,6,7,8-penta-	$H_3 = 7.52$; $H_4 = 7.61$; $H_9 = 8.38$	$J_{3,4} = 8.85$	Kuroki et al., 1984
1,3,4,6,7-penta-	$H_2 = 7.51$; $H_8 = 7.53$; $H_9 = 8.11$	$J_{8,9} = 8.4$	Kuroki et al., 1984
1,3,4,6,8-penta-	$H_2 = 7.48$; $H_7 = 7.54$; $H_9 = 8.14$	$J_{7,9} = 2.0$	Bell and Gara, 1985
1,3,4,6,9-penta-	$H_2 = 7.51$; $H_7 = 7.44$; $H_8 = 7.34$	$J_{7,8} = 8.9$	Bell and Gara, 1985

TABLE 2-8 (cont.)

PCDF	Chemical Shifts (ppm downfield from tetramethylsilane)	Coupling Constants (J) (Hertz)	Reference
1,3,4,7,8-penta-	H_2 = 7.46; H_6 = 7.73; H_9 = 8.31 H_2 = 7.49; H_6 = 7.77; H_9 = 8.36		Bell and Gara, 1985 Kuroki et al., 1984
1,3,4,7,9-penta-	H_2 = 7.51; H_6 = 7.56; H_8 = 7.44	$J_{6,8}$ = 1.9	Bell and Gara, 1985
2,3,4,6,7-penta-	H_1 = 7.89; H_8 = 7.47; H_9 = 7.68 H_1 = 7.93; H_8 = 7.50; H_9 = 7.71	$J_{8,9}$ = 8.4 $J_{8,9}$ = 8.2	Bell and Gara, 1985 Kuroki et al., 1984
2,3,4,6,8-penta-	H_1 = 7.92; H_7 = 7.54; H_9 = 7.77	$J_{7,9}$ = 2.0	Kuroki et al., 1984
2,3,4,6,9-penta-	H_1 = 8.32; H_7 = 7.45; H_8 = 7.30	$J_{7,8}$ = 8.4	Bell and Gara, 1985
2,3,4,7,8-penta-	H_1 = 7.91; H_6 = 7.77; H_9 = 7.97 H_1 = 8.43; H_6 = 8.09; H_9 = 8.46 H_1 = 7.93; H_6 = 7.73; H_9 = 7.87		Kuroki et al., 1984 Safe and Safe, 1984 Bell and Gara, 1985
1,2,3,4,6,7-hexa-	H_8 = 7.55; H_9 = 8.16 H_8 = 7.53; H_9 = 8.14	$J_{8,9}$ = 8.25 $J_{8,9}$ = 8.5	Kuroki et al., 1984 Bell and Gara, 1985
1,2,3,4,6,8-hexa-	H_7 = 7.59; H_9 = 8.22 H_7 = 7.59; H_9 = 8.21	$J_{7,9}$ = 1.8 $J_{7,9}$ = 1.9	Kuroki et al., 1984 Bell and Gara, 1985
1,2,3,4,6,9-hexa-	H_7 = 7.50; H_8 = 7.40 H_7 = 7.48; H_8 = 7.38	$J_{7,8}$ = 8.7 $J_{7,8}$ = 8.5	Kuroki et al., 1984 Bell and Gara, 1985
1,2,3,4,7,8-hexa-	H_6 = 7.79; H_9 = 8.41 H_6 = 7.77; H_9 = 8.40		Kuroki et al., 1984 Bell and Gara, 1985
1,2,3,4,7,9-hexa-	H_6 = 7.59; H_8 = 7.48 H_6 = 7.58; H_8 = 7.47	$J_{6,8}$ = 1.8 $J_{6,8}$ = 1.9	Kuroki et al., 1984 Bell and Gara, 1985
1,2,3,4,8,9-hexa-	H_6 = 7.68; H_7 = 7.52	$J_{6,7}$ = 8.8	Bell and Gara, 1985
1,2,3,6,7,8-hexa-	H_4 = 7.74; H_9 = 8.35 H_4 = 7.72; H_9 = 8.34 H_4 = 8.16; H_9 = 8.51		Kuroki et al., 1984 Bell and Gara, 1985 Safe and Safe, 1984
1,2,3,6,7,9-hexa-	H_4 = 7.73; H_8 = 7.55		Bell and Gara, 1985
1,2,3,6,8,9-hexa-	$H_{4,7}$ = 7.71 or 7.74		Kuroki et al., 1984
1,2,3,7,8,9-hexa-	$H_{4,6}$ = 7.65		Bell and Gara, 1985
1,2,4,6,7,8-hexa-	H_3 = 7.65; H_9 = 8.36 H_3 = 7.99; H_9 = 8.54		Kuroki et al., 1984 Safe and Safe, 1984

TABLE 2-8 (cont.)

PCDF	Chemical Shifts (ppm downfield from tetramethylsilane)	Coupling Constants (J) (Hertz)	Reference
1,2,4,6,7,9-hexa-	H_3 = 7.71; H_8 = 7.57 H_3 = 7.69; H_8 = 7.54		Kuroki et al., 1984 Bell and Gara, 1985
1,2,4,6,8,9-hexa-	$H_{3,7}$ = 7.73 H_3 = 8.02		Kuroki et al., 1984 Norstrom et al., 1979
1,3,4,6,7,8-hexa-	H_2 = 7.52; H_9 = 8.30 H_2 = 7.51; H_9 = 7.51		Kuroki et al., 1984 Bell and Gara, 1985
2,3,4,6,7,8-hexa-	$H_{1,9}$ = 7.92 $H_{1,9}$ = 7.91 $H_{1,9}$ = 8.24 $H_{1,9}$ = 8.32		Kuroki et al., 1984 Bell and Gara, 1985 Norstrom et al., 1979 Safe and Safe, 1984
1,2,3,4,6,7,8-hepta-	H_9 = 8.35		Kuroki et al., 1984
1,2,3,4,6,8,9-hepta-	H_7 = 7.75		Kuroki et al., 1984
1,2,3,4,7,8,9-hepta-	H_6 = 7.74		Kuroki et al., 1984

The 2-carboxylic acid of DBF has been made by the Grignard reaction from 2-MBDF. Since BDBFs yield the lithio compounds by interchange, all four lithio-DBFs are accessible and the lithium atoms can be replaced by carboxyl, methyl (using dimethyl sulfate), halogen or amino groups (using O-methylhydroxylamine).

OFDF reacts with sodium methoxide in methanol to yield the 3-mono- and 3,7-diether. Similarly, 1,2,3,4-TFDF undergoes nucleophilic substitution reactions with sodium hydrogen sulfide, sodium methiolate and lithium tetrahydridoaluminate, in which the 3-fluorine atom is replaced by SH, SMe and H, respectively.

The directing effects of functional groups at the 2-position appear to be mainly toward the 8-position regardless of whether the group is electron-attracting or electron-withdrawing (Keumi et al., 1972a). This is not so for the 3-position since 3-halo- groups give substantial amounts of the 7-substituted compounds (Table 2-9).

2-MIDF reacts with ammonia over cuprous bromide at 200-210°C to yield 95% 2-amino-DF (Brown and Coleman, 1973a). 3-MBDF directs the nitronium ion to the 1-position; 1-MBDF is nitrated at the 3-position (Grinev et al., 1973); the TrCDFs are monochlorinated by $SbCl_5/CCl_4$ reagent to produce TCDFs (Table 2-10).

2.4.2. Photodecomposition. Irradiation of various lower chlorinated PCBs in aqueous suspensions of 1 and 10 mg/ℓ at 254 nm (mercury arc) caused no appearance of PCDFs. Irradiation at 310 nm resulted in PCDFs from 2,5-dichloro- and 2,2',5,5'-tetrachloro-PCB to the extent of ~0.2% after irradiation times of up to 200 hours. This suggests that PCDFs may be destroyed at 254 nm but only very slowly at 310 nm (Hutzinger et al., 1973). The pres-

TABLE 2-9

Friedel-Crafts Acetylation of Substituted PHDFs*

PHDF	Conditions	Acetylating Agent	Derivative	Percentage Yield
-(2-Br)	Nitrobenzene/$AlCl_3$ (50-60°C/1 hr)	Benzoyl chloride	8-	84
-(2-Cl)			8-	84
-(3-Br)			8- 7-	56 31
-(3-Cl)			8- 7-	60 3

*Source: Keumi et al., 1972a

TABLE 2-10

Chlorination of TrCDF with Antimony Pentachloride/CCl_4 Reagent to Produce TCDFs*

Produced TCDF	Original TrCDF																											
	123	124	126	127	128	129	134	136	137	138	139	146	147	148	149	234	236	237	238	239	246	247	248	249	346	347	348	349
1234	X	X					X									X												
1236	X		X					X									X											
1237	X			X					X									X										
1238	X				X					X									X									
1239	X					X					X									X								
1246		X	X									X									X							
1247		X		X									X									X						
1248		X			X									X									X					
1249		X				X									X									X				
2346																X	X				X				X			
2347																X		X				X				X		
2348																X			X				X				X	
2349																X				X				X				X
1346							X	X				X													X			
1347							X		X				X													X		
1348							X			X				X													X	
1349							X				X				X													X
1267			X	X																							X	X
1268			X		X																		X	X				
1269			X			X								X	X													
1278				X	X														X	X								
1279				X		X				X	X																	
1289					X	X																						
1367								X	X																	X		X
1368								X		X												X		X				
1369								X			X		X		X													
1378									X	X								X		X								
1379									X		X																	
1467												X	X												X			X
1468												X		X							X			X				
1469												X			X													
1478													X	X			X			X								
2367																	X	X								X	X	
2368																	X		X			X	X					
2378																		X	X									
2467																					X	X			X		X	
2468																					X		X					
3467																									X	X		

*Source: Adapted from Mazer et al., 1983a,b ence of the triplet sensitizer, 4,4'-dichlorodibenzophenone, increased photodecomposition of 2,8-DCDF (Crosby and Moilanen, 1973).

Choudhry and Hutzinger (1982a,b) reviewed the photochemical formation and degradation of PCDFs. PCDFs could be produced in yields up to 10% from polychlorinated diphenyl ethers (0.8 mM in methanol) bearing 2- or 2'-chloro substituents after 88 hours irradiation at 310 nm (Choudhry et al., 1977a,b). The addition of the triplet sensitizer acetone at 0.45 M markedly increased the PCDF yield while suppressing reductive dechlorination. Greater than 50% yields of 2-MCDF, 2,3-DCDF and 2,3,8-TrCDF were obtained in the presence of acetone at 310 nm after 10-15 hours irradiation of 2,4'-dichloro-, 2,4,5-trichloro- and 2,4,4',5-tetrachlorodiphenyl ether, respectively. The PCDFs can also be produced in solvents such as n-hexane and ethanol (Choudhry and Hutzinger, 1982a,b).

The photodegradation of OCDF irradiated at 310 nm in n-hexane is more rapid than that of 2,8-DCDF similarly irradiated (Hutzinger et al., 1972a,b, 1973). The half-lives were ~1.5 and 2.5 hours, respectively. There was also a pronounced solvent effect. The half-life for OCDF in methanol was ~15 minutes. This is the reverse of results with OCDD (Crosby et al., 1971). Irradiation of both PCDFs in hexane for 20 hours caused formation of a "yellow gum," probably a polymer, which showed no recognizable chlorine isotopic patterns in the mass spectra. At shorter irradiation times, reductive dechlorination was postulated to be the major photoprocess although no photoproducts were identified unambiguously. When water was used as a solvent and thin films of the two PCDFs were irradiated at 310 nm, the same products were formed although much more slowly. The 2,8-DCDF also produced a TrCDF, probably because the viscous film allowed free radical "cages" to exist for comparatively long times. This proves that •Cl is produced during the irradiations. The OCDF in a thin film or in solution appeared to produce PCDFs to HpCDFs.

Crosby and Moilanen (1973) confirmed the previously stated results for 2,8-DCDF (10-100 mg/ℓ) in methanol after irradiation in a solar simulator and in addition, unambiguously identified 2-MCDF as the first reductive dechlorination product. As expected, the half-life was longer than reported for OCDF above (~75 hours). The 2-MCDF was almost unaffected by further irradiation (the half-life was ~1 day), whereas 95% of the 2,8-DCDF was degraded within 48 hours. The photodecomposition rate depended also on the purity of the methanol, technical grade methanol containing acetone (a triplet sensitizer) decreasing substrate half-life to near 25 hours. The absence of lower chlorinated biphenyls in most environmental samples was thought to indicate a potential for the occurrence of the hydroxylation and PCDF photochemical routes in the environment (Crosby et al., 1973).

Buser (1976b) reported that 4 hours of UV irradiation (mercury arc) of 25 μg/mℓ OCDF in 95% hexane/benzene caused extensive dechlorination, ~90% of the compound being degraded. The major products were HxCDFs and HpCDFs with trace amounts of PeCDFs. After 24 hours of irradiation, HxCDFs predominated (40%) along with PeCDFs and HpCDFs, and also trace amounts of TCDFs. Only 2% of OCDF remained. At least 13 HxCDFs were formed from the prolonged irradiation. The dechlorination was not as selective as for OCDD. These events were also confirmed by Firestone (1977b). Preferential reductive dechlorination (mostly to HpCDFs) was induced by 4 hours of γ-irradiation (^{60}Co; 1.4 Mrad/hr). Unfortunately, specific isomers were not identified in any of these experiments.

Mazer and Hileman (1982) and Mazer et al. (1983a,b) irradiated eight TCDFs (2,3,6,8-, 2,4,6,7, 1,2,7,8-, 1,4,6,8-, 1,2,4,8-, 2,4,6,8-, 2,3,7,8-

and 1,2,3,4-) at 1 μg/mℓ in n-tetradecane or n-hexane with 254 nm light for 4 hours. GS/MS after HPLC showed the TrCDFs were mostly formed (Table 2-11). DCDFs were also produced towards the end of the photolysis experiments. The order of photodegradation for chlorines in 1,2,3,4-TCDF was 3- > 2- > 1- = 4-. For 1,2,4,8-TCDF, the 1- and 2-positions were most reactive. Thus, it was found that chlorines on the same aromatic ring stabilize the loss of a chlorine from that ring, that adjacent chlorines around a given chlorine will facilitate preferential cleavage of that chlorine, and that if there are an equal number of vicinal chlorines, the 3-chlorine will be lost before the 2-chlorine.

Mazer and Hileman (1982) and Hileman et al. (1985) also reported on the photodegradation of PeCDFs (1,2,3,6,7-, 1,2,3,4,8-, 2,3,4,6,9-, 1,3,4,6,7-, 2,3,4,6,8- and 2,3,4,6,7-) at 254 nm in hexane (Table 2-12). The same general principles appear to hold as for the TCDFs, that is, the most substituted ring tends to lose chlorine, especially if adjacent chlorines are present. A quantitative analysis of the data is not yet available.

Ballschmiter et al. (1985) showed that the dechlorination of OCDF by UV light favored expulsion of the chlorine in the 9-position and to a lesser extent the 8-position. The 1,8-congeners dominated among the HpCDF and HxCDFs formed.

2.4.3. Pyrolysis. Very few studies of pyrolysis of pure PHDFs have been reported. Thermal degradation of OCDF at 400°C for 15 hours in a sealed silica tube resulted in expulsion of chlorine in the 9-position (Ballschmiter et al., 1985), and OCDF was thermally much less stable than OCDD. HpCDFs mainly resulted with the 1,2,3,4,6,7,8-HpCDF dominating.

TABLE 2-11

Photodegradation of TCDFs (1 μg/mℓ) at 254 nm After 4 Hours of Irradiation in Hexane or Tetradecane[a]

	TrCDFs and Their Relative Ratios[b] Formed by Photolysis			
TCDF	Major	Second	Third	Fourth
4:0[c]				
1,2,3,4-	1,2,4- 1.0	1,3,4- 0.38	2,3,4- 0.13	1,2,3- 0.12
3:1				
1,2,4,8-	2,4,8- 1.0	1,4,8- 0.4	1,2,8- 0.1	1,2,4- ND
2:2				
2,3,6,8-	2,4,8- 1.0	2,4,7- 0.59	2,3,8- 0.09	2,3,6- 0.03
1,4,6,8-	2,4,6- 1.0	1,4,6- 0.59	2,4,9- 0.60	1,4,8- 0.21
2,4,6,8-	2,4,8- 1.0	2,4,6- 0.40	-	-
2,4,6,7-	2,4,7- 1.0	2,4,6- 0.45	3,4,8- 0.1	3,4,6- ND
2,3,7,8-	2,3,8- 1.0	2,3,7- 0.52	-	-
1,2,7,8-	1,2,8- 1.0	2,3,8- 0.94	1,2,7- 0.75	2,3,9- 0.37

[a] Source: Mazer and Hileman, 1982

[b] Assuming equivalent mass spectrometer responses.

[c] 4:0 Substitution indicates four chlorines on one aromatic ring and no chlorines on the other aromatic ring.

ND = None detected at the point at which DCDFs began to form.

TABLE 2-12

Photodecomposition of PeCDFs in Hexane at 254 nm*

Produced TCDF	Original PeCDF																											
	12346	12347	12348	12349	12467	12468	12469	12478	12479	12367	12368	12369	12378	12379	12389	12489	13467	13468	13469	13478	13479	13489	23467	23468	23469	23478	23479	12678
1234	X	X	X	X																								
1236	X									X	X	X																
1237		X								X			X	X														
1238			X								X		X		X													
1239				X			X					X		X	X													
1246	X				X	X	X																					
1247		X			X			X	X																			
1248			X			X		X								X												
1249				X			X		X							X												
2346	X																						X	X	X			
2347		X																					X			X	X	
2348			X																					X		X		X
2349				X																					X		X	X
1346	X																X	X	X									
1347		X															X			X	X							
1348			X															X		X		X						
1349				X															X		X	X						
1267					X					X												X						X
1268						X					X					X												X
1269							X					X				X						X						
1278								X					X		X													X
1279									X					X	X							X						
1289															X	X												

TABLE 2-12 (cont.)

Produced TCDF	Original PeCDF																											
	12346	12347	12348	12349	12467	12468	12469	12478	12479	12367	12368	12369	12378	12379	12389	12489	13467	13468	13469	13478	13479	13489	23467	23468	23469	23478	23479	12678
1367											X							X				X						X
1368										X		X							X									X
1369										X			X							X		X						
1378														X	X						X							X
1379															X							X						
1467						X												X		X						X		
1468							X												X						X	X		
1469								X												X								
1478									X				X								X					X		
2367											X										X			X			X	
2368									X			X													X		X	
2378														X													X	
2467						X													X					X	X			
2468							X																		X			
3467																		X						X				

*Source: Adapted from Mazer et al., 1983a,b

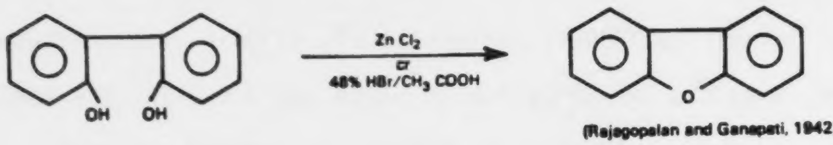
2.5. SYNTHESIS

Synthesis of all 135 isomers in large quantities is still an important research priority.

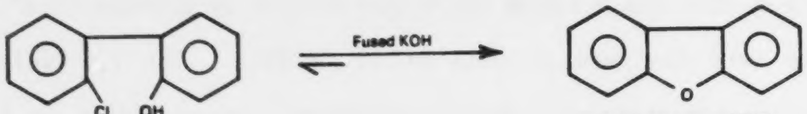
2.5.1. General Methods. Coffey (1973) has eviewed the older general synthetic methods leading to the production of PCDFs.

PHDFs are produced by the following methods:

a. Eliminating water or hydrogen halide from substituted 2,2'-dihydroxy- or 2-halo-2'-hydroxybiphenyls, respectively:


OH
OH
Zn Cl2
or
48% HBr/CH3 COOH
O

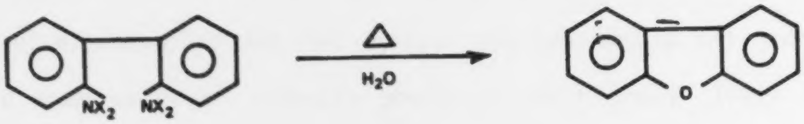
(Rajagopalan and Ganapati, 1942)


Cl
OH
Fused KOH
O

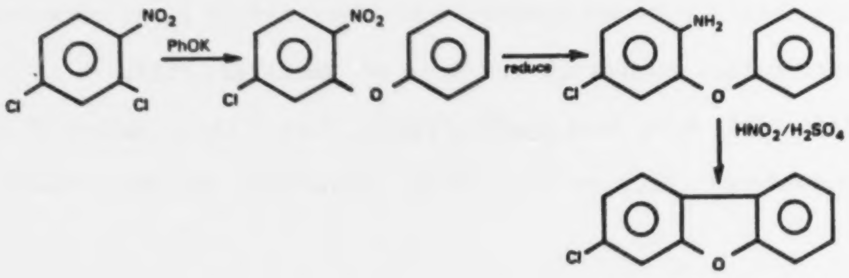
(Schimmelschmidt, 1950)

Both types of reactions occur more easily under alkaline conditions when halogen groups are present ortho- or para- to the hydroxy and halogen groups to be eliminated (Alphen, 1932; Case and Schock, 1943):

b. Decomposing bisdiazonium salts of substituted 2,2'-diaminobiphenyls, although phenazones are frequent contaminants (Coffey, 1973):


NX2
NX2
Δ
H2O
O

c. Diazotization of substituted o-aminobiphenyl ethers (McCombie et al., 1931):


NO2
Cl
Cl
PhOK
NO2
Cl
O
reduce
NH2
Cl
O
HNO2/H2SO4
Cl
O

where Ph = C_6H_5-

d. PHDFs have been prepared in poor yield by the pyrolysis of substituted diphenyl ethers (Choudhry and Hutzinger, 1982a,b) and chlorophenols (Bell, 1936). This route may be of environmental significance since incineration is often used to dispose of phenol-impregnated woods and papers (Chapter 4). The reaction of phenol and HCl at 550°C for 5 minutes for an initial HCl concentration >1 mM caused the production of trace amounts of PCDFs and PCDDs (Eklund et al., 1986).

2.5.2. **Methods for PHDFs.** Gilman et al. (1934a) reacted dibenzofuran and chlorine in carbon tetrachloride to yield 30% 2,8-DCDF. The 2-MCDF was produced in a similar fashion in ethanol as solvent at 60°C. The 2-MCDF, 3-MCDF-, 2,8-DCDF and 3,8-DCDF were synthesized by Shibata et al. (1952) and procedures were reviewed by Oita et al. (1955). The 1,3-cycloaddition of 3,4,5,6-tetrachlorobenzene-2-diazo-1-oxide in chlorobenzene at 130°C yields 1,2,3,4-TCDF (Huisgen et al., 1964).

The presence of higher PCDFs as reaction by-products was noted by Clark (1940) after the chlorination of dibenzofuran, and also by Cox et al. (1965) after vacuum pyrolysis of various dibenzofuran-based polymers and copolymers. Cox et al. (1965) observed that the chlorinated polymers were more stable than the brominated ones except for the tetra-brominated ones. Ried and Eng (1969) found that o-quinone diazides added benzene with nitrogen elimination to yield the 1,2,3,4-TCDF, 1,2,3-TrCDF and 1,3-DCDF. OCDF was produced by pyrolysis of pentachlorophenol (Sanderman et al., 1958).

Further interest in the synthesis of PCDFs had to await more interest in their toxicological properties and those of commercial PCBs.

Specific PCDFs have been synthesized by direct chlorination of unsubstituted dibenzofuran (Gray et al., 1976), photolysis of chlorinated diphenyl ethers (Choudhry et al., 1977a,b; Norstrom et al., 1976a, 1977; Lindahl et al., 1980), by Ullman reaction (Morita et al., 1977b), photodechlorination of OCDF (Buser, 1976b) and by thermal oxidative cyclization of specific PCB isomers (Morita et al., 1978; Buser et al., 1978a,d; Buser and Rappe, 1979), or chlorobenzenes (Buser, 1979). The mechanisms involved in the thermochemical generation of PCDFs have been reviewed by Choudhry and Hutzinger (1982b). Nearly all of these methods result in complex mixtures which require extensive chromatography and purification before pure compounds can be obtained, in general, with final yields <10%.

A synthetic route with environmental implications is the thermal production of PCDFs from PCBs. Buser et al. (1978a,d) and Buser and Rappe (1979) showed that when specific PCBs were pyrolyzed in quartz ampules between 500 and 700°C, PCDF yields in the 1-10% range could be obtained, though they were accompanied by many other products, including chlorinated benzenes, naphthalenes and hydroxy PCBs. Buser et al. (1978a) described the products of pyrolysis at 600°C, identified by GC/MS. Yields were as high as a few percent in favorable cases. The decompositions are summarized in Table 2-13. There appear to be four major paths for production of PCDFs from PCBs: loss of two ortho chlorines, loss of ortho hydrogen as well as chlorine, loss of an ortho hydrogen as well as chlorine but involving a shift of chlorine from the 2- to the 3-position and loss of two ortho hydrogens. These paths are summarized in Figure 2-1 for 2,2',4,4',5,5'-hexachlorobiphenyl. Such paths are environmentally important in the origin of toxic PCDFs and PBDFs from various PCB and PBB formulations, respectively. Mazer and Hileman (1982), Mazer et al. (1983a,b) and Hileman et al. (1985) have utilized the technique to produce 110 pure TCDFs to act as chromatographic standards after HPLC separation and confirmation by GC/MS and Kovats indices.

TABLE 2-13

Formation of PCDFs from the Pyrolysis of Specific PCBs[a]

PCB Pyrolyzed	PCDFs Formed
Tetra:	
2,3,4,5-	2,3,4-tri-[b]; 1,2,3,4-tetra-
2,3,5,6-	1,2,4-tri-
2,6,2',6'-	1,9-di; 1,4,9-tri-
Penta:	
2,3,4,5,6-	1,2,3,4-tetra-[b]; small amounts of 1,2,4-tri-; 2,3,4-tri-;
2,4,5,2',5'-	2,3,8-tri-[b]; small amounts of 2,3,6,8-tetra- plus three other tetra; 1,3,4,6,9-penta-;
2,4,5,3',4'-	2,3,7,8-tetra-[b]; 2,3,6,7-tetra-; 1,3,4,7,8-penta;
2,4,6,2',4'-	1,3,7-tri-[b]; 1,3,6,7-tetra-; 1,3,7,9-tetra-; 1,3,4,7-tetra-;
2,3,6,2',5'-	1,4,8-tri-; 1,2,8-tri-; 1,4,6,8-tetra-; 1,2,6,8-tetra-; 1,2,4,8-tetra-[b]; 1,2,6,9-tetra-;1,4,6,9-tetra-;
Hexa:	
2,4,5,2',4',5'-	2,3,7,8-tetra-[b]; 2,3,4,7,8-penta-; 1,3,4,7,8-penta
2,4,6,2',4',6'-	1,3,7,9-tetra; 1,3,4,7,9-penta-;
2,4,5,2',4',6'-	1,3,7,8-tetra-[b]; 1,3,4,7,8-penta-; 1,3,4,7,9-penta
2,3,4,2',3',4'-	3,4,6,7-tetra-[b]; 1,2,3,6,7-penta-:
2,3,5,2',3',5'-	2,4,6,8-tetra-; 1,2,4,6,8-penta-;
2,3,6,2',3',6'-	1,2,4,8-tetra-[b]; 1,2,8,9-tetra-[b]; 1,2,4,6,9-penta(?); 1,2,4,8,9-penta-;
3,4,5,3',4',5'-	2,3,4,6,7,8-hexa-; small penta-

TABLE 2-13 (cont.)

PCB Pyrolyzed	PCDFs Formed
Hepta:	
2,3,4,5,2',3',4'-	2,3,4,6,7-penta-; 1,2,3,4,6,7-hexa-[b]; 1,2,3,6,7,8-hexa-; 1,2,3,4,7,8,9-hepta-; 1,2,3,4,6,7,8-hepta-; 1,2,3,4,6,7,9-hepta-;
2,3,4,5,2',4',5'-	2,3,4,7,8-penta-[b]; 1,2,3,4,7,8-hexa-; 1,3,4,6,7,8-hexa-[b]; 2,3,4,6,7,8-hexa-; 1,2,3,4,6,7,9-hepta-;
Octa:	
2,3,4,5,2',3',4',5'-	2,3,4,6,7,8-hexa-[b]; 1,2,3,4,6,7,8-hepta-[b]

[a] Source: Buser and Rappe, 1979

[b] Most abundant PCDF

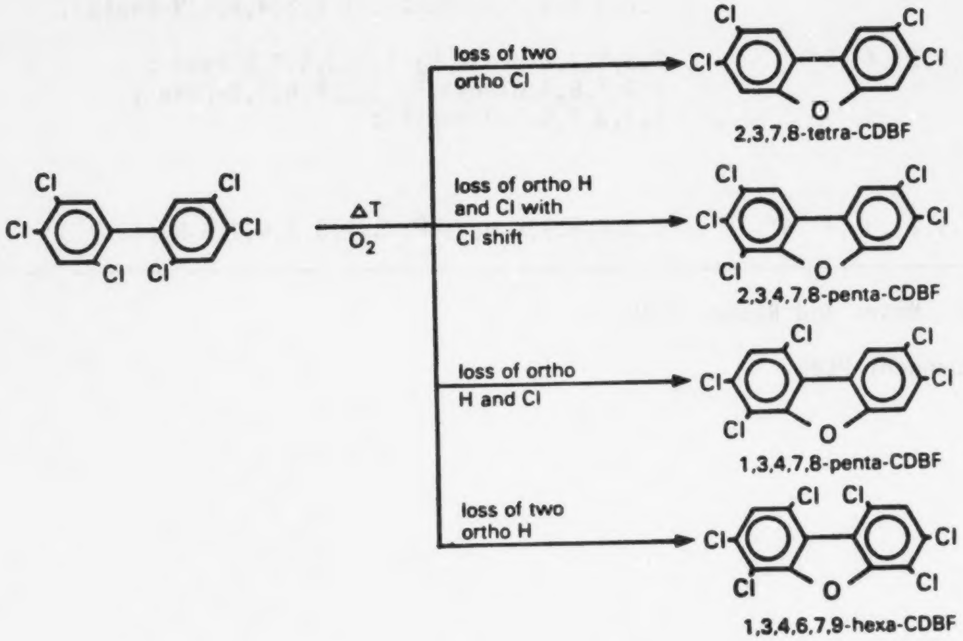

Cl
Cl
Cl
Cl
Cl
Cl
ΔT
O2
loss of two ortho Cl
2,3,7,8-tetra-CDBF
loss of ortho H and Cl with Cl shift
2,3,4,7,8-penta-CDBF
loss of ortho H and Cl
1,3,4,7,8-penta-CDBF
loss of two ortho H
1,3,4,6,7,9-hexa-CDBF
O

FIGURE 2-1

Possible Reaction Schemes to Produce PCDFs from the Pyrolysis of 2,2', 4,4', 5,5'-Hexachlorobiphenyl

Source: Adapted from Buser and Rappe, 1979

The formation of PCDFs by pyrolysis of commercial chlorobenzenes is similarly important (Buser, 1979). PCDDs, chlorinated naphthalenes, chlorinated styrenes and chlorophenols are also produced. Samples that were pyrolyzed at 620°C in quartz ampules in the presence of air contained the PCDF levels shown in Table 2-14. The PCDF content was much higher than the PCDD content in all cases, especially for the trichlorobenzenes for which the ratio of PCDF to PCDD content was >40. The amounts of the toxic PCDF isomers (2,3,7,8-TCDF-; 1,2,3,7,8-PeCDF-; 2,3,4,7,8-PeCDF-) were small.

Lindahl et al. (1980) reported that pyrolysis of 2,2',4,4',5,5'-hexachlorodiphenyl ether in quartz ampules at 500, 600 and 700°C for 1 minute yielded 0.1, 1.3 and 0.1%, respectively, of PCDFs. The products were 1,2,4,6,8,9-HxCDF, 1,2,4,7,8-PeCDF and some PCDDs. More PCDFs than PCDDs were formed when chlorodiphenyl ethers were pyrolyzed under these conditions. Between 3.5 and 4.5% PCDFs were produced when the 3,5,4'-tri-, 2,4,5,4'-tetra-, 2,4,6,3',5'-penta- and 2,3,4,5,6,2',3',4'-octa-chloro-diphenyl ethers (CDPEs) were pyrolyzed at 600°C compared with up to 0.6% of PCDDs. PCDF production was not so favored from the hexa-chlorodiphenyl ethers (2,3,4,2',3',4'-, 2,3,4,2',3',5'-, 2,3,4,2',4',5'- and 2,4,5,2',4',5'-), PCDF and PCDD contents both ranging from 0.8-1.3%. The same mechanisms depicted in Figure 2-1 for production of PCDFs from PCBs also prevail from chlorinated diphenyl ethers except that loss of 2-ortho-H, ortho-H and -Cl are favored. Loss of two ortho-H appears to be favored for the tri- and tetra-CDPEs, but loss of ortho-H and -Cl is predominant for higher CDPEs. The octa-CDPE also yielded HxCDFs that can be formed when two chlorines are lost by rearrangement, one of which was the 1,2,3,4,6,7-HxCDF formed by loss of two ortho-Cl. Buser (1986) found that pyrolysis of three polybrominated diphenyl ethers used as flame retardants resulted in yields

TABLE 2-14

Formation of PCDFs from Pyrolysis of Chlorobenzenes*

Chlorobenzene Compound	Total Amount Pyrolyzed (μg) (ratio of isomers)	Percentage of PCDFs Formed Relative to Chlorobenzene				
		Tetra-	Penta-	Hexa-	Hepta-	Octa-
Tri- (1,2,3-; 1,2,4-; 1,3,5-)	200 (1:1:1)	0.20	0.55	0.28	0.025	$<2.5\times10^{3}$
Tetra-	200	$<10^{-4}$	2.5×10^{-3}	0.080	0.23	0.10
Penta-	200	$<10^{-4}$	$<2.5\times10^{-3}$	$<2.5\times10^{-3}$	$<2.5\times10^{-3}$	$<1.5\times10^{-3}$
Mixture (tri-; tetra-; penta-)	500 (1:1:1)	0.040	0.12	0.22	0.12	1.20×10^{-4}

*Source: Recalculated from Buser, 1979

of PBDFs and PBDDs of up to 10% at 510-630°C in quartz ampules. The mechanism of formation appeared to be the same as that for the chlorinated derivatives. The 2,3,7,8-TBDF was not a major product from pyrolysis of penta-, octa- or decabromodiphenyl ethers.

Perchlorination has also been investigated as a synthetic route to the PCDFs (Williams and Blanchfield, 1972; Huckins et al., 1974; Crist and Moseman, 1977; Ballschmiter et al., 1985). The perchlorination of 2-hydroxybiphenyl with thionyl chloride/antimony pentachloride/iodine produced 8% of pure OCDF (Gara et al., 1980). No lower PCDFs were formed from the reaction. Perchlorination with BMC Reagent ($SO_2Cl_2/S_2Cl_2/AlCl_3$ is facile (Ballschmiter et al., 1985). OBDF cannot be synthesized because of steric hindrance though HpBDF can (Richtzenhain and Schrage, 1978).

The previous methods do not produce high enough yields, and require too extensive chromatography to be useful for synthetic purposes in animal experiments. Methods producing higher yields of PCDFs are now available, however, using chlorinated diphenyl ethers as reaction intermediates. Thus, 3,7-DCDF, 2,3,8-TrCDF and 2,3,7,8-TCDF could be produced in 43-47% yields (Table 2-15). Norstrom et al. (1979) and Gara et al. (1979) have refined the technique so that yields of 30-84% can be easily achieved in a single synthetic step without having to separate a very complex mixture (Table 2-15). Typically, 2.5 mmol of the diphenyl ether and 5 mmol of palladium diacetate were refluxed in 50 mℓ acetic acid with 5.6 g methanesulfonic acid. After the reaction (Table 2-15), the acetic acid was evaporated off, the mixture made basic (solid NaOH), extracted twice with chloroform or carbon tetrachloride, filtered, dried ($MgSO_4$) and analyzed by GC/MS. Some of the PCDFs had to be further purified by crystallization, column chroma-

TABLE 2-15

Synthetic Methods for PCDFs Using Chlorinated Diphenyl Ethers (CDPEs) as Substrates

Starting CDPE	PCDF	Reaction Time (hours)	Yield (%)	Recrystallizing Solvent	Reference
3',4,4'-Tri-	2,3,8-Tri-	0.50	46	n-Hexane	Sanderman et al., 1958
3,3',4,4'-Tetra-	2,3,7,8-Tetra-	1.0	43	n-Hexane	Sanderman et al., 1958
3,3'-Di-	3,7-Di-	0.66	47	MeOH	Norstrom et al., 1977
2,2',4,4',5,5'-Hexa-	1,2,4,6,8,9-Hexa-	14	36	Chloroform	Norstrom et al., 1977
2,2',3,3',4,4'-Hexa-	2,3,4,6,7,8-Hexa-	3.5	55	Chloroform	Norstrom et al., 1977
2,2',4,4'-Tetra-	2,4,6,8-Tetra-	2.0		CCl_4	Gara et al., 1979
2,2',4,4',5-Penta-	1,2,4,6,8-Penta-	2.0	67	CCl_4[a]	Crist and Moseman, 1977
2,3',4,4',5-Penta-	1,2,4,6,8-Penta-	2.0	54	CCl_4[a]	Crist and Moseman, 1977
2,2',3,4,4'-Penta-	2,3,4,6,8-Penta-	2.0	49	CCl_4[a]	Crist and Moseman, 1977
2,2',3,4,4',5-Hexa-	1,2,4,6,7,8-Hexa-	2.0	84	CCl_4[a]	Crist and Moseman, 1977
2,2',3,3',4,4',5-Hepta-	1,2,3,4,6,7,8-Hepta-	2.0	33	CCl_4	Crist and Moseman, 1977

[a] With column chromatography

[b] With preparative TLC

tography or preparative-TLC. Bell and Gara (1985) have synthesized all 87 TCDFs, PeCDFs, HxCDFs, HpCDFs and OCTA using this synthetic route.

Another method is the cyclization of diazotized chlorophenoxy-o-anilines (Gray et al., 1976; Kuroki et al., 1984). Commonly, a nitro-chlorinated diphenyl ether is obtained by reaction of the appropriate potassium halophenolate and the halonitrobenzene at 120°C for 15 hours. The nitro group is converted to an amino group (CH_3COOH/Fe for 15 hours), diazotized and cyclized with isoamyl nitrite in tetrachloroethylene (80°C for 15 hours). The crude product is purified by column chromatography and recrystallized. Overall yields are typically ~6-10%.

A modification of the above is the base-catalyzed cyclization of hydroxy-PCBs in DMSO/KOH (Safe and Safe, 1984). The hydroxy-PCB can be prepared from coupling of a chlorinated anisidine and 1-substituted symmetrical benzene at 120°C in isoamyl nitrite for 18 hours followed by purification and demethylation (BBr_3/CH_2Cl_2 at 20°C for 24 hours). It can also arise from coupling of a 2-chloro-aniline with a chlorinated anisole in isoamyl nitrite followed by purification and demethylation. The yields including the initial coupling reaction range from 3-15%. The major advantage of the latter modification is that only single isomers are usually produced.

Bell (1983) has reported on the synthesis of uniformly labeled ^{13}CTCDF, using the Ullman ether procedure or by phenoxide displacement on an iodonium salt. Yields commonly exceeded 30%. Synthesis of ^{14}C-2,3,7,8- TCDF was first reported by Gray (1981).

Pyrolysis of octafluorodibenzothiophene-5,5'-dioxide affords perfluorodibenzofuran (Chambers et al., 1968).

1,2,3,4-TFDF is prepared from hexafluorobenzene and o-lithioanisole. The o-methoxy biphenyl was first formed, then the free phenol (by reaction with aluminum chloride) and finally the TFDF by refluxing in with potassium carbonate and dimethylformamide (Brown et al., 1967).

3. SAMPLING AND ANALYTICAL METHODS

3.1. SUMMARY

The major thrust of research in analytical methods for the PHDFs has been toward specific synthesis, separation and sensitive detection of certain toxic PCDFs and PBDFs, especially the 2,3,7,8 derivatives. One such analytical method is the capillary-GC/MS technique developed by scientific teams headed by Buser and Rappe (1979); many groups have now used this technique.

Earlier, the lack of analytical standards severely hampered the quantitation and identification of specific isomers, though methods of quantitating the total isomers containing a given number of halogens (GC/MS) and total PCDFs (by perchlorination) have been developed. Since many compounds have been shown to co-elute with PCDFs on gas chromatographic columns, and compounds (for example, chlorinated diphenyl ethers) may interfere with the mass spectral analysis, relatively specific cleanup techniques have been developed, often based on the use of aluminum oxide column chromatography and judicious choice of solvents, usually various combinations of n-hexane and methylene dichloride. Techniques have been developed to identify PHDFs in PCBs, Yusho oil, chlorophenols, fly ash, Agent Orange, 2,4,5-T, wood dust, animal tissues, gelatin, human and fish tissues, mothers' milk and bovine milk, wastewater and PBBs. Other methods are not as sensitive or as specific as capillary-GC/MS. Of some utility as a screening test before GC/MS is a radioimmunoassay technique. A cytosol receptor bioassay also shows promise.

Sampling of PHDFs is comparatively underdeveloped. Charcoal and XAD-2 resin have been used to sample vapors of PCDFs. Modified EPA-5 sampling trains are used to sample stack emissions.

3.2. SAMPLING METHODS

Few sampling methods have been developed specifically for PHDFs since interest has been focused on the analysis of various environmental and commercial samples obtained by the usual procedures. A review has been presented by Tiernan (1983). The federal procedures governing custody, safety, transportation, data review, sampling and the analysis of PCDDs and PCDFs have been summarized by Elly (1985).

3.2.1. Incinerators.

PCDFs and PCDDs in the vapor phase have been collected by adsorption onto charcoal (Norit Pk, 0.5-1 mm or Amberlite XAD-2, 20/50 mesh) contained in a glass filter (Rappe et al., 1978b). The vapors were drawn through a glass funnel into the filter by means of a vacuum pump. Both adsorbents had to be cleaned by prior Soxhlet extraction with methylene dichloride for 24 hours. The PCDFs and PCDDs were desorbed by Soxhlet extraction with methylene dichloride for 10 hours. Particulate PCDD or PCDF can be trapped on glass wool filters that can be eluted with toluene (Ahling et al., 1977). PCDFs and PCDDs in the vapor phase were also collected after this filter by adsorption onto Chromosorb W coated with 30% Apiezon M; the columns can be eluted with hexane and toluene. PCDF and PCDD particulates and vapors have also been collected in impingers similar to a U.S. EPA method 5 sampling train (Olie et al., 1977, 1982; Cavallaro et al., 1980; Gizzi et al., 1982; Redford et al., 1983; Wang et al., 1983; Benfenati et al., 1983; Chiu et al., 1983; Taylor et al., 1983; Ballschmiter et al., 1984; Brocco et al., 1984; Clement et al., 1985a; Haile et al., 1985; Weerasinghe et al., 1985a; Scheidl et al., 1985). Only three groups of investigators have attempted to characterize the collection of PCDFs in the segments of their sampling train. The closest approximation to the EPA-5 sampling train (Clement et al., 1985a) was the addition of two Florisil (10 g) cartridges after the third impinger. In general, 95% of the total PCDD/PCDF was found in the impingers, ~5% on the filter before the first impinger, and 0.3% on the Florisil cartridges. The backup Florisil cartridge contained 5% of PCDF found in the front cartridge. The volatile PCDFs were relatively enriched on the Florisil filter compared with the prefilter and impingers. However, the extraction recovery of spiked 1,2,3,4-TCDD, 2,3,7,8-TCDD, 2,3,7,8-TCDF, a PeCDD, a HxCDD, a HpCDD and the OCDD averaged only 43%.

In another sampling train (Ballschmiter et al., 1984), the sequence of components was a glass fiber filter, a condenser at 15-18°C, and two impingers containing methoxyethanol at 0°C. The PCDD/PCDF ratio on the filter and in the condensate were similar, but PCDFs were much more concentrated than PCDDs in the impingers with no HpCDD/HpCDF and OCDD/OCDF present here. This confirmed the vapor phase nature of the condensable fraction. The overall collection efficiency was not determined.

The sampling train utilized by Tiernan et al. (1985) comprised, in order, a heated sampling probe, a heated cyclone, a heated glass fiber filter, and a series of eight impingers at 0°C, the first two containing water and with XAD-2 traps intervening between empty impingers 3, 4 and 5. Sodium arsenite solution was placed in impingers 5 and 6, impinger 7 was empty, and impinger 8 was charged with silica gel. Known amounts of 1,2,3,4-TCDD (1.3 μg) and 1,2,4,8-TCDF (1 μg) were vaporized and collected with 92 and 97% efficiency, respectively. Most of the PCDDs and PCDFs (~62%) were collected in two XAD-2 traps. The recovery of spiked $^{37}Cl_4$-2,3,7,8-TCDD was <80% for the acetone back rinse, the cyclohexane back rinse and the front XAD-2 trap.

The sampling method recommended by the Swedish EPA is also based on a modified EPA-5 train (Beryvall and Jansson, 1986). The sampling equipment is fortified before sampling with known amounts of ^{13}C-surrogate compounds in the filter, condensate or adsorbent (XAD-2) to act as internal standards.

Fly ash samples have been collected from different points in flue gas streams by electrostatic precipitation (Buser et al., 1978b). Since the highest PCDD and PCDF levels were found from samples collected at flue gas temperatures of 200-260°C, gas stream temperature should be measured during sampling. Procedures to assure representative analysis of the collected samples have not been developed. It is obviously desirable, however, to analyze as much of the sample as possible. Soot type samples are generally sampled with Kleenex tissues (Rappe et al., 1983c).

Liquid samples should be thoroughly shaken or ultrasonicated before cleanup and analysis. If liquid samples contain solid matter, it is probably advisable to filter the solution and perform analysis on the filtrate and retain the dry solid. The choice of solvent to elute any adsorbed PCDFs depends on the nature of the solid. Probably methylene dichloride will be adequate for most situations; recovery tests should be performed to choose the correct solvent.

Organ and tissue samples are usually preserved in 4 or 10% buffered formalin (Nagayama et al., 1977). Both tissue and formalin should be analyzed for PCDFs. Another much-used alternative is to freeze the tissues at -70°C (Albro et al., 1985).

3.3. GC METHODS FOR THE PCDFs

3.3.1. Packed Column GC. Because certain PCDF isomers are extremely toxic to animals (Chapter 7), interest in the analysis of these compounds has increased. Much of the early work was hampered by losses incurred during sample cleanup and analysis, and by the fact that the PCDF peaks often coincided with the peaks of chlorinated diphenyl ethers containing two more chlorine atoms (Crummett and Stehl, 1973). Thus, a confirmative spectroscopic technique, such as mass spectroscopy using multiple ion monitoring, must be used to prove the specificity of the separation. Quantitation without such confirmation will yield maximum possible values of the contaminant rather than the true value. The contaminant must be identified before valid quantification can occur. These problems have been reduced by the use of capillary GC, which gives more specific separation.

The presence of PCDFs in PCBs was first deduced by Vos et al. (1970), on the basis of GC/MS evidence. A 2 mg sample of PCB in hexane was placed on a Florisil column and eluted with hexane followed by 5% ethyl ether in hexane until all PCBs were eluted. The PCDFs were eluted with 25% ether in hexane and the eluate was evaporated. The residue was then chromatographed on a Pyrex column (5 ft x 1/8 in) containing 10% DC 200 on Gas Chrom Q (80/100 mesh) held at 200°C, with nitrogen as carrier gas at 50 mℓ/min and the separated peaks subjected to mass spectroscopy. Only the Clophen A-60 and Phenoclor DP-6 PCBs contained PCDFs since they were the only ones that caused a toxic response in the chick edema assay. The investigators could not separate or identify the specific isomers involved. A similar procedure was also utilized by Bowes et al. (1973) to determine PCDFs in PCBs. Before GC/MS analysis, however, the three eluates described in Vos et al. (1970) were subjected to further fractionation on an aluminum oxide column with an initial elution with 1% methylene chloride/hexane to collect the PCBs and then with 20% methylene chloride/hexane to elute the PCDFs. Bowes et al. (1973) also analyzed the eggs, fat and liver of sea gulls for PCDF content. Samples were freeze-dried, ground with sodium sulfate (2:1) in a heavy glass mortar, extracted using the Soxhlet procedure with a hexane/acetone azeotrope, the solution concentrated and cleaned up by passing over sulfuric acid-impregnated Celite before concentrating and continuing the cleanup on the Florisil column mentioned in the Vos method. No account of recoveries were given.

The lack of analytical standards for the PCDFs hampered further analysis, but still did not prevent Roach and Pomerantz (1974), Bowes et al. (1975a,b), Nagayama et al. (1975, 1976) and Kuratsune et al. (1976) from establishing the presence of PCDFs in many PCBs, in Kanemi oil and in Yusho patients exposed to Kanemi oil, from the mass spectrum alone, without standards. Morita et al. (1977a) postulated the presence of the 2,3,7,8-TCDF on the basis of GC retention-time data for an authentic sample, but the levels quoted were maximal because of the relatively low resolving power of the packed GC column used. Morita et al. (1977a) eluted the activated Florisil column with hexane and then 95:5 hexane/acetone to remove the PCBs and collected the PCDFs by eluting with pure acetone. Recoveries were >90%. This procedure had also been recommended by Vos et al. (1970). Morita et al. (1977a) then used the MS technique to quantitate the PCDFs using the parent ions.

The analysis of PCDFs in chlorophenols also was similarly impeded by lack of authentic standards. Nevertheless, PCDFs were found in chlorophenols (Firestone et al., 1972; Plimmer et al., 1973; Schwetz et al., 1974a,b; Buu-Hoi et al., 1972) in the crude base-insoluble organic extract of the chlorophenol. Buu-Hoi et al. (1972) showed that consideration of the entire spectrum allowed discrimination from other interfering compounds, whereas reliance on specific ions led to errors. For example, the mass spectrum of 2,2',3,3',4,4',5,5',6-nonachloro-6'-hydroxydiphenyl ether showed loss of two chlorine atoms to give a peak that coincides with the molecular ion of HxCDFs (m/e 372). The presence of such ethers can be verified by methylation and subsequent GC/MS (Plimmer et al., 1973). Typically (Firestone et al., 1972), chlorophenol was converted to the phenolate by reaction with sodium hydroxide, the PCDFs extracted in petroleum ether, the organic layer washed, concentrated and transferred to an aluminum oxide column, the PCDFs being eluted by the original procedure of Vos et al. (1970) plus a final diethyl ether elution. The concentrates were then partitioned between petroleum ether and sulfuric acid, the organic layer neutralized with sodium bicarbonate and the solutions evaporated and taken up in isooctane for GC/MS and EC/GC analysis. Recovery of 2,3,7,8-TCDD was 33%; omission of the sulfuric acid step increased recovery to 63%. No recoveries were cited for PCDFs. Firestone et al. (1972) used a 6 ft x 1/4 inch Pyrex column packed with 5% OV-101 on 80/100 mesh Chromosorb W (HP) under temperature programing at 10°C/min to 250°C. The sulfuric acid step was subsequently eliminated (Plimmer et al., 1973). Capillary GC/MS is discussed in Section 3.4.2.3.

Villanueva et al. (1974) found HpCDFs and OCDF in hexachlorobenzene by using aluminum oxide column chromatography before GC/MS and using the cleanup technique of Firestone et al. (1972). The Pyrex GC column (10 ft x 6.3 mm OD) was packed with 3% SE-30 on 80/100 mesh Chromosorb W (AW/DMCS) at 230°C, using a helium carrier (70 mℓ/min). The injector temperature was 230°C. The recovery of the OCDF from the cleanup procedures was ~72%, much lower than for the OCDD.

Similarly, PCDFs were identified in 2,4,5-T and Agent Orange. The 2,4,5-T ester formulation was diluted with chloroform and placed on a PX-21 foam-charcoal column. The nonpolar components in the formulation were eluted with chloroform. Moderately polar compounds were then eluted with benzene. The PCDDs and PCDFs were finally recovered by elution of the column with 1:1 toluene/benzene and then the eluate concentrated before aluminum oxide chromatography. The aluminum oxide column was washed with petroleum ether before the concentrate was placed on the column. The PCDDs and PCDFs were eluted with 1:4 methylene dichloride/petroleum ether. The GC column was a 0.91 m x 4 mm ID Pyrex column packed with OV-7 on Chromosorb W (HP) at 200°C. The temperature of the injector was 250°C. The average recovery for the 2,3,7,8-TCDD was >91%. Although the presence of PCDFs was inferred from the MS data, again no specific isomer identification was made and no recoveries of PCDFs found (Huckins et al., 1978). Ahling et al. (1977) used the same technique but eluted the PCDDs and PCDFs with toluene and confirmed the existence of PCDFs using the available authentic samples and standards.

PCDF standards were probably first used by Hutzinger et al. (1973) and by Crosby et al. (1973) to identify the reductive dechlorination products of 2,8-DCDF irradiated in hexane solution by UV light. DCDFs, TrCDFs, TCDFs and OCDF were not found in various tissues of wildlife in the Bay of Fundy/ Gulf of Maine area by Zitko (1972). Tissue samples were ground in anhydrous sodium sulfate and Soxhlet extracted with hexane; the solution was evaporated and applied to an aluminum oxide column. The column was eluted with hexane, the effluent concentrated and then applied to a silicic acid column, eluted with hexane and then with 10% ether/hexane. The hexane eluate was further chromatographed on aluminum oxide and then eluted by 20% methylene dichloride/hexane. The 6 ft x 4 mm GC column was packed with 4% SE-30 on Chromosorb W (AW; 60/80 mesh) at 250°C. The estimated recoveries of DCDFs, TrCDFs and TCDFs were each 94%, and that of OCDF was 83% over the spiking range of 0.23-1.41 ng/g. These column chromatographic conditions, when compared with those cited previously, appear too mild to produce such good recoveries.

3.3.2. Representative Sample Preparations and Methods of Analysis.

3.3.2.1. INTRODUCTION -- In this section, the various optimized analytical treatments of selected samples will be presented with an indication of how the method evolved. In all the methods, the final step is GC/MS. Thus, the mass spectrometric characteristics of the PCDFs are discussed first, ending with the various treatments for selected samples.

Reviews of the analytical methodology to determine PCDFs have been authored by Crummett (1983), Albro and Parker (1980), Tiernan (1983), Rappe et al. (1983a), Buser (1985) and Buser et al. (1985). A step-by-step procedure for extraction/cleanup/GC-MS of a typical sample is provided by Taylor et al. (1983). Albro et al. (1985) have presented a system to ensure a valid interlaboratory comparison of PCDFs and PCDDs in human adipose tissue.

3.3.2.2. MASS SPECTROMETRY OF CHLORODIBENZOFURANS --Mass spectrometry, using an electron impact source, involves the fragmentation of a molecule into positively or negatively charged ions (usually singly charged) that are collected quantitatively so that the resultant plot of normalized ion current versus mass/charge ratio is an identifying fingerprint for the compound. The technique can be made quantitative by external and internal standards techniques.

3.3.2.2.1. Chlorine Isotope Patterns -- Before discussing the mass spectra of PCDFs, it will be helpful to review the patterns of ion intensities that result from the two chlorine isotopes at 35 and 37 amu. Since these isotopes have natural abundances of 75.5 and 24.5%, respectively (a ratio of 3.08), any ion containing one chlorine will give peaks in the mass spectrometer at two mass numbers separated by two mass units; if the intensity of the first peak is 100%, the intensity of the second (higher) mass peak will be 32% (100%/3.08). If more than one chlorine atom is present, the binomial distribution is convoluted with the isotopic abundance to give the appropriate peak pattern. Table 3-1 shows the relative intensity distributions for ions containing 1-8 chlorines. Thus, by matching a pattern in an unknown mass spectrum with a pattern given in Table 3-1, it is usually possible to determine the number of chlorine atoms present in an unknown chlorinated aromatic compound.

3.3.2.2.2. **Mass Spectra of PCDFs** -- The mass spectra of PCDFs are dominated by the presence of many chlorine isotope patterns and by an abundant molecular ion. In fact the molecular ion is the most intense peak in the mass spectrum. The only fragmentation of the PCDFs is the loss of up to three chlorine atoms and the loss of CO (Buser, 1975). The fragment ions are summarized in Figure 3-1, which also gives the average relative intensity of each ion. Each ion is accompanied by the isotopic cluster of peaks, each of which has a relative intensity dependent on the number of chlorines in it, their relative intensities given by the values in Table 3-1.

The complete mass spectra of TrCDFs to OCDF have appeared in the literature; they are given in Figure 3-1. For example, HxCDF has an intense molecular ion cluster at m/e 372. By convention the mass of the lightest mass peak in an isotopic cluster is referred to as the mass of that ion. The loss of one and two chlorines gives peaks at m/e 337 and 302. The loss of CO from each of these ions gives peaks at m/e 309 and 274, and the loss of another chlorine from the latter gives an ion at 239. The ion caused by the loss of CO-Cl is the daughter ion used in MS-MS (Ryan et al., 1985a,b,c).

TABLE 3-1

Relative Intensities of Ions Containing from One to Eight Chlorine Atoms

(ions of relative intensity 1% or less have been omitted)

Number of Chlorines	Mass of Fragment	Relative Intensity, %
1	M	100
	M + 2	32
2	M	100
	M + 2	65
	M + 4	10
3	M	100
	M + 2	97
	M + 4	31
	M + 6	3
4	M	77
	M + 2	100
	M + 4	49
	M + 6	11
5	M	62
	M + 2	100
	M + 4	65
	M + 6	21
	M + 8	3
6	M	51
	M + 2	100
	M + 4	81
	M + 6	35
	M + 8	8
7	M	44
	M + 2	100
	M + 4	97
	M + 6	52
	M + 8	17
	M + 10	3
8	M	34
	M + 2	88
	M + 4	100
	M + 6	65
	M + 8	26
	M + 10	7

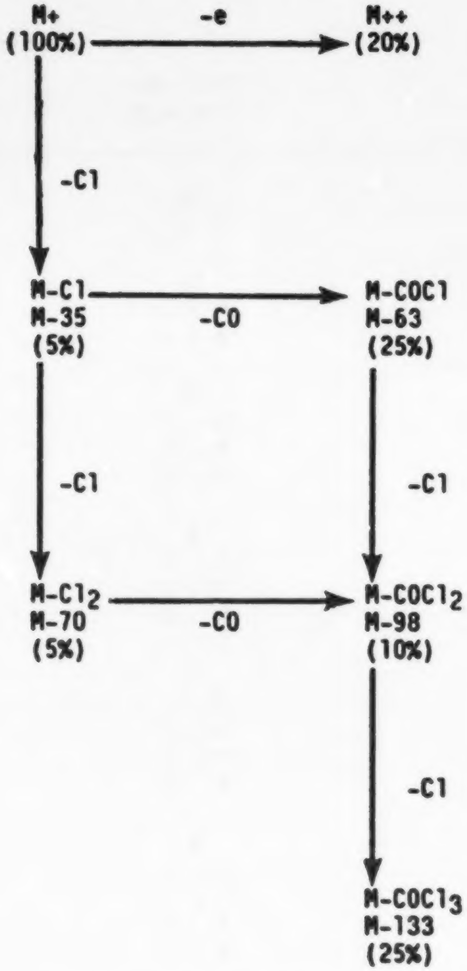

M+
(100%)
-e
M++
(20%)
-Cl
M-Cl
M-35
(5%)
-CO
M-COCl
M-63
(25%)
-Cl
-Cl
M-Cl2
M-70
(5%)
-CO
M-COCl2
M-98
(10%)
-Cl
M-COCl3
M-133
(25%)

FIGURE 3-1

Mass Spectral Behavior of PCDFs
Percent Relative Intensities of Each Ion Are Given in Parentheses

Source: Buser, 1975

The isotopic patterns associated with each of these ions agrees with the expected number of chlorine atoms as given by the relative intensities of the clusters in Figure 3-1; for example, the cluster at m/e 239-245 has peaks of relative intensities characteristic of three chlorines (see Table 3-1). Since three chlorines have been lost from a molecule containing six chlorine atoms to form this ion, it should indeed have a 3-chlorine pattern.

The fragment ions of the MCDFs to OCDF are summarized in Table 3-2. In addition, the number of chlorine atoms in each ion is given in parentheses so that the appropriate isotopic pattern can be noted. Clearly, mass numbers of ions characteristic of the various PCDFs can be specified from data such as these. In addition, using such data, PCDFs can be identified with accuracy in environmental samples.

3.3.2.2.3. Mass Spectra of Isomeric PCDFs -- Characterizing isomeric PCDFs by electron impact MS alone is not possible with present technology (Buser, 1985). For example, 2,3,8-TrCDF and 2,4,6-TrCDF have identical mass spectra (Gray et al., 1976). This is to be expected if the fragmentation mechanisms operating in these two compounds are not sensitive to the positions of the chlorine atoms. Table 3-2 summarizes the intensities of the four major ions of various PCDFs (Gray et al., 1976). All isomers, except 2,3,6,8-TCDF, regardless of the position or number of chlorine atoms, have the same losses of COCl and $COCl_3$ from their molecular ions within an experimental error of ±20% of the observed peak intensity. For 2,3,6,8-TCDF, the reported intensities of the M-COCl and $M-COCl_3$ ions are much greater than for the other compounds. The spectrum should be remeasured. Parker et al. (1983) have shown that the molecular ion carries 52±5% of the total ion current for PeCDFs to OCDF in magnetic sector MS.

TABLE 3-2

Percent Intensities of the Major Ions in the Mass Spectra of Isomeric PCDFs[a]

	M^+	M-COCl	$M-COCl_3$	M^{++}
$2,3,8-Cl_3$	100	23	14	11
$2,4,6-Cl_3$	100	23	14	11
$2,3,7-Cl_3$	100	25	24	22
$2,3,9-Cl_3$	100	25	22	20
$2,3,6,8-Cl_4$	100	41[b]	56[b]	23
$2,4,6,8-Cl_4$	100	22	22	22
$2,3,7,8-Cl_4$	100	19	16	12
$1,2,7,8-Cl_4$	100	17	22	20
$1,2,4,7,8-Cl_5$	100	22	26	-
$1,3,4,7,8-Cl_5$	100	18	22	24
Average	100	22 ± 3	20 ± 4	18 ± 5

[a] Source: Adapted from Gray et al., 1976

[b] Outliers

Even though MS alone cannot distinguish among isomers, GC can separate many of them from one another. In fact, many GC retention values for specific PCDF isomers have been published (Section 3.3.2.3.).

3.3.2.2.4. **High Resolution Mass Spectrometry of PCDFs** -- Using double focusing mass spectral instrumentation, it is possible to measure the mass of an ion to 6 or 7 significant figures. Because the various isotopes have masses that differ slightly from their nominal value (i.e., chlorine-35 has a mass of 34.9689 amu), such high mass accuracy permits calculation of the exact elemental composition of an ion from its exact mass. The exact molecular weights of the various PCDFs are given in Table 3-3.

Because of the high aromaticity and the large number of chlorine atoms, these exact masses are up to 0.25 amu below the normal mass (see $C_{12}Cl_8O$). Thus, these ions can easily be distinguished from an ion at the same nominal mass that may come from a relatively saturated compound. For example, at m/e 440, a hydrocarbon ion may be $C_{32}H_{56}$, which has an exact mass of 440.4382; this is 0.6924 amu higher than the corresponding OCDF molecular ion. When taken together with the chlorine isotopic patterns and adequate chromatographic separation (see Table 3-1), these exact masses (Table 3-3) are almost specific indicators of the presence of PCDFs in samples. The chlorinated diphenyl ethers lose 2 chlorines in the ion source of a mass spectrometer to form a compound with the same elemental composition as a PCDF. The molecular ion of the ether must be monitored to prove whether the interference is present or not.

3.3.2.2.5. **Specific Ion Monitoring Mass Spectrometry** -- Buser (1975) based his quantitation of PCDFs in commercial chlorophenols on selected ion monitoring of the molecular ions (304, 338, 372, 406 and 440; see Table 3-2). Figure 3-2 is an example of such data; the peaks attributable to the

TABLE 3-3

Exact Molecular Weights of the PCDFs
(masses used to calculate these molecular weights:
C = 12.000000, H = 1.007825, Cl = 34.968855 and O = 15.994915)

Compound	Molecular Weight
$C_{12}H_7ClO$	202.0185
$C_{12}H_6Cl_2O$	235.9796
$C_{12}H_5Cl_3O$	269.9406
$C_{12}H_4Cl_4O$	303.9016
$C_{12}H_3Cl_5O$	337.8627
$C_{12}H_2Cl_6O$	371.8237
$C_{12}HCl_7O$	405.7847
$C_{12}Cl_8O$	439.7458

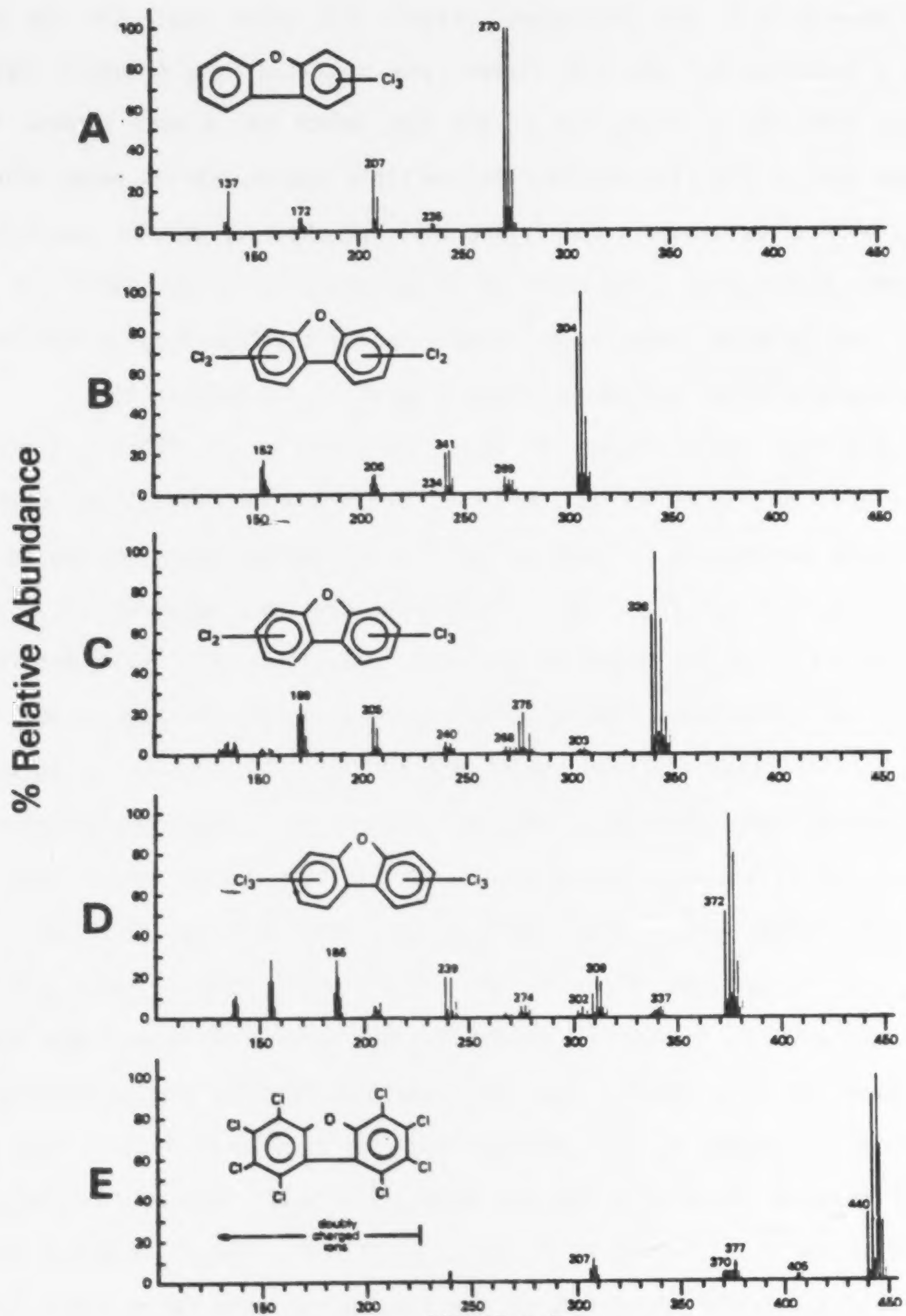


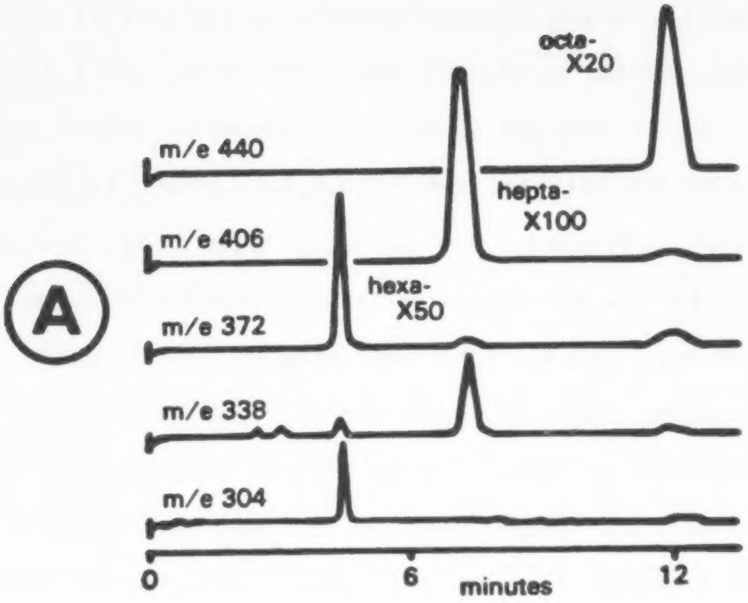
FIGURE 3-2

Low resolution mass spectra of (A) TrCDF, (B) TCDF, (C) PeCDF, (D) HxCDF and (E) OCDF. In all cases the chlorine substitution pattern is not known. The fragmentation patterns are explained in Figure 3-1.

Source: (A) Hites and Lopez-Avila, 1979; (B,C,D) Bowes et al., 1973; (E) Hutzinger et al., 1972b.

HxCDF through OCDF are indicated. Figure 3-3 shows that m/e 372 (Cl_6) gives a response for the OCDF isomer (see retention time 12 min). This is because OCDF has an $M-Cl_2$ ion at m/e 370, which has a more intense first isotope peak of 372. The specific and multiple ion monitoring modes allow pg sensitivity to be attained and is the best technique to use for quantitation of PCDFs after prior separation on a capillary GC column (Buser et al., 1985). For example, Rappe et al. (1977) showed that the 2,3,7,8-TCDF isomer was probably present in Yusho oil (see Figure 3-3 and Chapter 4).

3.3.2.2.6. Other Types of Mass Spectroscopy of PCDFs -- Electron-impact (EI) MS (50-70 eV at 250°C) is the preferred analytical mode for TCDFs with sensitivity 1-10 pg by specific ion monitoring. Decreasing sensitivity occurs for the higher chlorinated species (Rappe et al., 1983a; Buser et al., 1985). Negative chemical ionization (NCI) with methane as reagent gas (0.35 torr, 180°C) is very sensitive (1-2 orders of magnitude more sensitive than electron impact MS) for all PCDF from tetra- to octa-, but requires more cleaning of the ion source, or a specially designed ion source, and is not as reproducible as an electron impact source (Buser et al., 1985; Rappe et al., 1983c; Hass et al., 1981; Kuehl et al., 1981). The base peak is usually M^- with (M^-+H•-Cl•) ions, both singly and multiply charged. As in electron impact MS, NCI spectra of isomers are identical (Buser et al., 1985). The ions used for specific ion monitoring are M or M^-+2. Rappe et al. (1983c) have reported that isomers have different response factors in the NCI mode. The NCI/EI response ratios range from 0.86 for 1,2,7,9-TCDD to 11 for 2,3,6,8-TCDF. The EI response is lowest for 1,2,6,7-TCDF being 59% of the highest response for a tetra-isomer, 1,3,6,8-. The NCI response is lowest for 1,2,7,9-tetrabeing 6.8% of that for the most responsive tetrachloro-isomer, 2,3,6,8-. For PeCDF, the



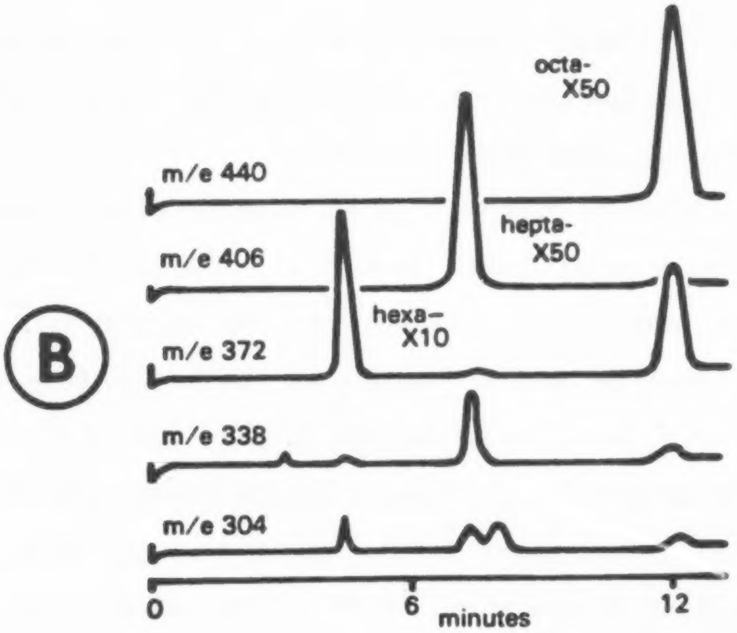


FIGURE 3-3

Mass chromatograms of (A) 2,3,4,6-tetrachlorophenol and (B) pentachlorophenol showing the elution of PCDF (m/e 304 = tetra-, m/e 338 = penta-, m/e 372 = hexa-, m/e 406 = hepta-, and m/e 440 = OCDF).

Source: Buser, 1975

2,3,4,6,8-isomer has an 81% response relative to the most EI responsive isomer, 1,2,4,6,8-; and 48% in the NCI mode. For HxCDF, the 1,2,3,4,7,8- isomer is 65% of the EI response of the most responsive isomer, 2,3,4,6,7,8-. The corresponding NCI data for the 1,2,3,4,7,8- isomer is 73% of the most responsive 1,2,4,6,7,8-isomer. For two hepta-isomers, the EI responses were equivalent, but for the NCI responses, the 1,2,3,4,6,8,9- isomer was 77% of the response of the 1,2,3,4,6,7,8-isomer (Rappe et al., 1983a). A Townsend discharge ion source (negative oxygen chemical ionization) seems to allow a better discrimination of isomers in each class (Hass et al., 1981; Chiu et al., 1983; Lao et al., 1985). Negative methane/oxygen chemical ionization is still being evaluated (Hass et al., 1981). The negative ion atmospheric pressure ionization mass spectrometry for 2,3,7,8-TCDF has been reported (Korfmacher et al., 1983). Though the detection limit was 0.5 pg, there are no significant mass spectral differences in the TCDF isomers having 4:0, 3:1 or 2:2 chlorine ring substitution patterns.

Another technique of promise is MS/MS (Voyksner et al., 1983; Sakuma et al., 1985; Shushan et al., 1985, Ryan et al., 1985a,b,c). The first analyzer provides nominal mass separation of the analyte ion (usually the molecular ion) from the matrix, the selected ion is then fragmented by collision (He, 2×10^{-4} mm Hg) into specific daughter ions (M-Cl, M-COCl) thus eliminating background noise. However, though cleanup problems are minimized, specific isomers cannot be distinguished through MS/MS alone, thus necessitating prior GC separation. MS/MS results appear to be consistently high with respect to GC/MS or GC/MS/MS for 2,3,7,8-TCDF (Voyksner et al., 1983). An MS/MS arrangement preceded by a short capillary column allows fast screening of TCDF congeners at 20-400 fg levels (Sakuma et al., 1985), with the SP-2340 column used for separating specific isomers.

3.3.2.3. CAPILLARY GAS CHROMATOGRAPHY -- The environmental chemistry groups headed by Buser, Bosshardt and Rappe have been largely responsible for the development of capillary GC techniques as applied to PCDFs. The GC/MS analyses noted in Section 3.3.2.1. were ultimately deficient because the various PCDF isomers were not resolved on packed GC columns, and often only the total PCDFs could be estimated. Since certain PCDFs are more toxic than others, separation of isomers is essential.

In view of the fact that each chlorinated class of PCDFs includes several congeners (with the exception of OCDF), the separation and quantitation of other isomers constituting that chlorinated class (for example quantitation of 2,3,7,8-TCDF in the presence of the 37 other TCDFs) is difficult. Conditions for isomer-specific quantitation are developed using calibration standards containing all of the isomers that may co-elute with the isomer of interest. A valid isomer-specific analysis will be accompanied by data (a mass chromatogram) that demonstrate the appropriate conditions that permit the separation of the isomer of interest from all other possible co-eluting isomers. If such calibration data is lacking, the identification of specific isomers must be regarded as tentative. Concentrations of isomers calculated on the basis of unvalidated isomer-specific data should be regarded as upper limits and therefore the concentrations are approximate.

Buser (1976a) was the first to use Pyrex capillary columns to produce the desired separation of the PCDF isomers. These columns with different stationary phases (OV-101, OV-17 and Silar 10C) allowed lower operating temperatures (205-225°C) as well as increased resolution compared with conventionally packed columns. The column characteristics are provided in Table 3-4. The average film thicknesses were 0.1 μm. The OV-101 is a nonpolar methyl silicone; the OV-17 is a semipolar methyl phenyl silicone;

TABLE 3-4

Characteristics of Some Glass Capillary Columns for Separating PCDFs*

Characteristic	Column OV-101	 OV-17	 Silar 10C
Length (m)	22	22	22
I.D. (mm)	0.32	0.34	0.34
Temperature (°C)	225	225	205
Retention time (OCDD) (min)	33.4	31.8	26.9
Retention index (OCDD)	3055	3420	3645
Theoretical plates	42,500	43,400	34,000
HETP (mm)	0.52	0.51	0.65
Carrier (atm)	He(0.5-0.6)	He(0.5-0.6)	He(0.5-0.6)

*Source: Buser, 1976a

and the Silar 10C is a highly polar cyanopropyl silicone. The capillary column was linked to the MS by a fused 0.15 mm ID platinum capillary at 250°C leading directly into the ion source of the MS. The compounds were detected by MS of the molecular ions. Isothermal conditions were found to be suitable for the separation of the PCDFs, and the reproducibility in quantitation was better than that obtained with temperature-programmed GC. The chromatography of Aroclor 1268 [which contains the most highly chlorinated PCBs (up to Cl_{10})], under the same conditions given in Table 3-4, produced complete PCB elution within 10 minutes on the Silar 10C column; the peaks were also better resolved than they were with a support-coated open tubular column. On this column, however, there was some overlapping of PCDD and PCDF isomers that did not occur on the others and there was also some reversal of elution order. When the same sample of PCDDs/PCDFs was injected on all columns, the OV-101 column always resolved more peaks. Better resolution of the less chlorinated isomers occurred on the OV-101 column, but the Silar 10C was best for the most highly chlorinated isomers. The OV-17 column gave the best resolution of the Cl_6 isomers. Relative retention times of PCDFs and PCDDs on these columns are presented in Table 3-5. All samples were introduced by an isothermal splitless technique.

Rappe et al. (1977) used the technique to analyze for PCDFs in Yusho oil but used 22 m x 0.36 mm ID glass capillary columns with OV-101 and OV-17 stationary phases and specific mass detection of the molecular ions (Figure 3-4). It was shown that the toxic 2,3,7,8-TCDF was probably one of the main PCDFs in Kanemi oil causing Yusho disease, constituting 30% of the total TCDFs. The 2,3,4,8-TCDF co-elutes with 2,3,7,8-TCDF on this column (Mazer et al., 1983a) and thus the identification is ambiguous. Buser et al. (1978a) used OV-17 capillary columns of 25 and 50 m length (0.36 mm ID) to

TABLE 3-5

Relative Retention Times of PCDFs and PCDDs on the Capillary Columns of Table 3-4*

	Relative Retention Times on Column		
Compounds	OV-101	OV-17	Silar 10C
TCDDs	0.13-0.18	0.10-0.15	0.12-0.22
TCDFs	0.10-0.14	0.09-0.12	0.11-0.19
PeCDDs	0.20-0.27	0.16-0.24	0.18-0.30
PeCDFs	0.17-0.24	0.14-0.23	0.15-0.25
HxCDDs	0.32-0.41	0.28-0.38	0.31-0.43
HxCDFs	0.29-0.42	0.25-0.40	0.24-0.50
HpCDDs	0.56-0.63	0.52-0.60	0.54-0.62
HpCDFs	0.51-0.65	0.47-0.66	0.44-0.66
OCDD	1.00	1.00	1.00
OCDF	0.978	0.025	0.933

*Source: Buser, 1976a

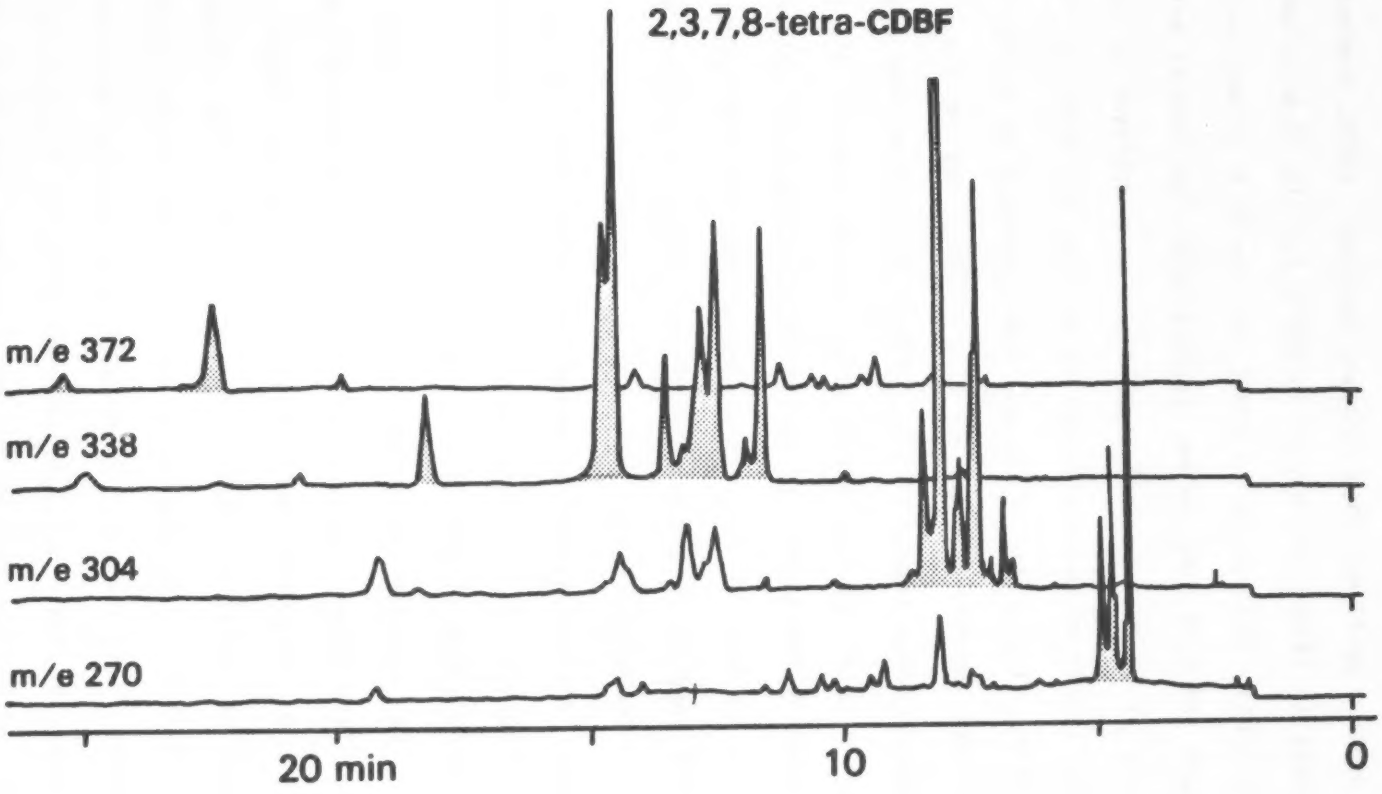


FIGURE 3-4

Mass Chromatograms (OV-101 Glass Capillary Column at 197°C) of "Yusho" Oil Showing Elution of PCDF (m/e 270 = TrCDF, m/e 304 = TCDF, m/e 338 = PeCDF and m/e 372 = HxCDF)

Source: Rappe et al., 1977

resolve the PCDF isomers produced by PCB pyrolysis, quantitating by GC/MS (Chapter 4). To achieve the best resolution, a complex temperature programming scheme to achieve splitless injection was adopted: 100°C, 2-minute isothermal; 15°C/minute to 145°C; 5°C/minute to 230°C for the 25 m column; 100°C, 2-minute isothermal, 20°C/minute to 230°C for the 50 m column, with helium carrier at 0.80 and 1.40 atmospheres, respectively. The samples were introduced splitlessly at 260°C. Optimal separations were achieved on the 50 m column. Similar techniques were utilized to analyze for PCDDs in fly ash (Buser et al., 1978b; Chapter 4). PCDDs and PCDFs produced by pyrolysis of chlorophenolates were detected (Rappe et al., 1978b), and in 2,4,5-T esters as well as Agent Orange (Rappe et al., 1978c). PCDFs have been analyzed in fly ash and in pyrolyzed PCBs (Buser et al., 1978c; Chapter 4); in Yusho oil and used Japanese PCB (Buser, et al. 1978d); in commercial chlorophenols (Rappe et al., 1978a); in the pyrolysates of specific PCB isomers (Buser and Rappe, 1979) and of chlorobenzenes (Buser, 1979); in tissues of patients who suffered from Yusho disease (Rappe et al., 1979b); in the products of the palladium acetate synthetic method for PCDFs (Gara et al., 1979); fish samples (Rappe et al., 1981); human adipose tissue, mothers' milk, bovine fat and bovine milk (Nygren et al., 1986).

Thus, it appears that optimum separations are achieved with a 50 m x 0.36 mm ID glass capillary column coated with OV-17, operated with helium carrier in the temperature programmed mode given by Rappe et al. (1978c). Sometimes a combination of capillary columns, however, is necessary. Rappe et al. (1984) reported that a 60m Supelco SP-2330 capillary column could separate most PCF isomers. The toxic 1,2,3,7,8-PeCDF does co-elute with the 1,2,3,4,8-isomer as does the toxic 1,2,3,4,7,8-HxCDF with the 1,2,3,4,7,9-isomer. These isomers can be separated on less polar columns like OV-17 and DB-5.

Farrell (1980) attempted unsuccessfully to produce the thinly coated columns mentioned previously (Rappe et al., 1978c). He did produce 25 m x 0.25 mm ID columns with film thicknesses of 0.4-0.5 μm OV-17 and OV-101, but analyses took 4-5 hours at 220-260°C and at 34 cm/sec linear velocity of nitrogen carrier. The problem may have been that the helium carrier was not used, since when the hydrogen carrier was used a mixture of DCDFs, TrCDFs, and TCDFs was resolved, the 2,3,7,8-TCDF being eluted in 6 minutes on a 50 m x 0.25 mm ID OV-101 column (0.5 μm liquid phase film) at 240°C and 100 cm/sec hydrogen carrier average velocity. Another deficiency of the Farrell (1980) study was that he did not use the splitless injection technique recommended by Buser (1976a). The splitless technique has now been successfully used by other laboratories (Mazer and Hileman, 1982; Mazer et al., 1983a,b; Hileman et al., 1985). Sources of PCDFs in the United States have been summarized by Cantrell et al. (1986).

The kinds of interferences expected in GC/MS of PCDFs are reviewed in Smith and Johnson (1983), Rappe (1984) and Lau et al. (1985). The quality assurance and control procedures to meet U.S. EPA requirements are embodied in RCRA method 8280 (Donnelly et al., 1986). Here a SP-2250 or equivalent column is recommended with multiple ion monitoring and internal standard quantitation. The peaks to be monitored include the following: TCDF, 319.897, 321.894, 327.885, 256.933 and 258.930; PeCDF, 353.858, 355.855; HxCDF, 389.816 and 391.813 for the PCDFs themselves, their (M-CO-Cl) ions, and the molecular ions for the interfering chlorinated diphenylethers as well as the molecular ions of chlorinated biphenylenes, PCBs and DDE isomers. All PCDF isomers should be on hand.

The most comprehensive recent list of retention times, flame ionization and electron capture sensitivities for TCDFs to OCDF is provided in Table 3-6 (Bell and Gara, 1985). Other lists can be found in Mazer and Hileman (1982); Firestone (1977a), Mazer et al. (1983a,b); Kuroki et al. (1984); Safe and Safe (1984) and Ligon and May (1984). Hale et al. (1985) have explained the retention behavior of all PCDFs in terms of number and position of chlorines, relations between chlorines, and ring interactions. Keith et al. (1983) have described a thermally modulated electron-capture detector that has higher sensitivity, better linear range and specificity to PCDFs than the conventional electron-capture detector.

3.3.2.4. TREATMENT OF SPECIFIC SAMPLES -- In the following methods, the methods were generally validated by the spiking of known amounts of PCDF and then measuring recovery. Such methodology does not necessarily measure efficiency if an initial pretreatment step is necessary to release PCDFs, as for fly ash samples. The quality assurance and control necessary to achieve valid results in the cleanup and column chromatographic steps are provided at length by Donnelly et al., (1986) in regard to an evaluation of RCRA Method 8280. The experimental conditions for elution of compounds from the "open" alumina microcolumn are critical. A 60% methylene dichloride in hexane-eluting solvent was much more reproducible than a 10% when neutral or basic alumina was used. Careful control of the water content was necessary since deactivation of neutral alumina caused weaker solvents to elute the PCDFs and PCDDs thus leading to losses and the presence of unwanted interferences.

3.3.2.4.1. PCB Formulations -- The PCB (100 mg) was placed on an aluminum oxide microcolumn (basic aluminum oxide, Woelm, in a disposable Pasteur pipette, 15 cm x 5 mm ID) and the bulk of the PCBs eluted with

TABLE 3-6

Relative Retention Times (RRT) and Relative Response Factors (RRF) for PCDFs Synthesized as Reference Standards[a,b]. The gas chromatographic detectors were flame ionization (FID) and electron capture (ECD).

Congener	Gas Chromatographic Column and Detector Used[c]					
	SP-2330	ECD	DB-5	FID	SP-2340	ECD
	RRT ±0.003	RRF ±0.05	RRT ±0.003	RRF ±0.05	RRT ±0.003	RRF ±0.05
1,2,3,4-	1.389	1.79	1.161	1.26	1.413	1.58
1,2,3,6-	1.415	0.81	1.150	0.70	1.452	0.83
1,2,3,7-	1.306	1.06	1.129	1.03	1.338	1.05
1,2,3,8-	1.396	1.00	1.148	0.87	1.446	0.99
1,2,3,9-	1.794	0.72	1.280	0.48	1.870	0.72
1,2,4,6-	1.290	0.51	1.201	0.85	1.313	0.46
1,2,4,7-	1.179	0.96	1.063	1.25	1.194	0.95
1,2,4,8-	1.258	1.15	1.100	1.12	1.283	1.24
1,2,4,9-	1.608	0.87	1.203	1.08	1.608	0.84
1,3,4,6-	1.252	0.70	1.070	1.06	1.278	0.68
1,3,4,7-	1.150	0.69	1.063	1.10	1.165	0.64
1,3,4,8-	1.217	0.84	1.080	1.01	1.244	0.78
1,3,4,9-	1.466	1.22	1.186	0.82	1.515	0.87
2,3,4,6-	1.949	0.61	0.201	0.85	2.074	0.60
2,3,4,7-	1.802	0.92	1.200	0.85	1.915	0.92
2,3,4,8-	1.900	0.97	1.197	0.96	2.006	0.93
2,3,4,9-	1.382	1.16	1.153	1.84	1.444	1.26
1,2,6,7-	1.561	0.79	1.189	1.49	1.637	0.76
1,2,6,8-	1.294	0.55	1.100	0.65	1.336	0.52
1,2,6,9-	1.854	0.78	1.258	0.85	1.953	0.74
1,2,7,8-	1.482	0.93	1.170	0.93	1.551	0.92
1,2,7,9-	1.569	0.83	1.208	0.89	1.633	0.93
1,2,8,9-	1.198	0.87	1.363	1.21	2.420	0.90
1,3,6,7-	1.188	0.63	1.079	0.88	1.216	0.60
1,3,6,8-	1.000	1.00	1.000	1.00	1.000	1.00
1,3,6,9-	1.314	1.05	1.123	1.13	1.345	1.10
1,3,7,8-	1.128	0.62	1.064	0.53	1.150	0.60
1,3,7,9-	1.131	0.84	1.084	0.70	1.145	0.82
1,4,6,7-	1.401	0.55	1.108	1.08	1.459	0.48
1,4,6,8-	1.163	0.75	1.026	1.10	1.189	0.77

TABLE 3-6 (cont.)

Congener	Gas Chromatographic Column and Detector Used[c]					
	SP-2330	ECD	DB-5	FID	SP-2340	ECD
	RRT ±0.003	RRF ±0.05	RRT ±0.003	RRF ±0.05	RRT ±0.003	RRF ±0.05
1,4,6,9-	1.586	0.94	1.152	1.30	1.643	0.91
1,4,7,8-	1.304	0.79	1.105	1.02	1.352	0.79
2,3,6,7-	1.978	0.72	1.234	0.93	2.128	0.73
2,3,6,8-	1.609	1.15	1.071	1.09	1.686	1.23
2,3,7,8-	1.866	1.05	1.198	1.15	1.989	1.02
2,4,6,7-	1.706	0.73	1.137	1.04	1.820	0.68
2,4,6,8-	1.390	0.67	1.035	0.86	1.447	0.74
3,4,6,7-	2.149	0.68	1.254	1.0	2.350	0.63
1,2,3,4,6-	1.997	3.94	1.573	0.67	1.186	3.87
1,2,3,4,7-	1.837	4.17	1.569	0.68	2.104	4.21
1,2,3,4,8-	1.961	7.27	1.596	1.25	2.262	7.25
1,2,3,4,9-	2.524	4.03	1.801	0.63	2.907	--
1,2,3,6,7-	2.058	2.90	1.636	0.54	2.391	3.05
1,2,3,6,8-	1.659	5.02	1.487	0.76	1.905	4.10
1,2,3,6,9-	2.394	4.31	1.726	0.72	2.758	3.74
1,2,3,7,8-	1.960	3.70	1.612	1.20	2.278	--
1,2,3,7,9-	2.023	2.24	1.664	0.34	2.331	1.84
1,2,3,8,9-	3.017	3.99	1.901	0.61	3.501	3.36
1,2,4,6,7-	1.809	3.84	1.493	0.87	2.087	4.12
1,2,4,6,8-	1.471	3.66	1.360	0.63	1.665	3.40
1,2,4,6,9-	2.111	4.77	1.569	0.74	2.419	4.10
1,2,4,7,8-	1.706	6.13	1.490	1.07	1.963	5.86
1,2,4,7,9-	1.759	4.64	1.533	0.77	2.005	3.98
1,2,4,8,9-	2.330	2.72	1.720	0.41	2.704	2.31
1,3,4,6,7-	1.760	2.67	1.491	0.81	2.041	2.94
1,3,4,6,8-	1.427	2.58	1.357	0.54	1.618	2.58
1,3,4,6,9-	1.923	3.76	1.544	0.71	2.213	3.20
1,3,4,7,8-	1.660	3.79	1.493	0.71	1.914	3.66
1,3,4,7,9-	1.615	5.43	1.506	1.33	1.843	4.40
1,3,4,8,9-	2.327	3.28	1.723	0.52	2.702	2.79
2,3,4,6,7-	3.074	3.79	1.746	0.81	3.709	4.04
2,3,4,6,8-	2.418	4.08	1.550	0.76	2.869	4.17
2,3,4,6,9-	1.843	3.10	1.512	0.64	2.149	3.07
2,3,4,7,8-	2.906	7.36	1.716	1.09	3.477	7.27
2,3,4,7,9-	1.568	3.50	1.474	0.66	1.806	3.45
2,3,4,8,9-	2.127	4.36	1.653	0.88	2.495	4.63

TABLE 3-6 (cont.)

Congener	Gas Chromatographic Column and Detector Used[c]					
	SP-2330	ECD	DB-5	FID	SP-2340	ECD
	RRT ±0.003	RRF ±0.05	RRT ±0.003	RRF ±0.05	RRT ±0.003	RRF ±0.05
1,2,3,4,6,7-	1.949	3.36	2.316	0.18	3.793	--
1,2,3,4,6,8-	2.320	2.42	2.066	0.57	2.943	2.35
1,2,3,4,6,9-	3.364	4.44	2.426	0.45	4.276	--
1,2,3,4,7,8-	2.800	6.14	2.320	0.54	3.592	--
1,2,3,4,7,9-	2.814	4.20	2.378	0.44	3.573	--
1,2,3,4,8,9-	4.251	4.84	2.771	0.34	5.426	--
1,2,3,6,7,8-	2.845	3.76	2.345	0.34	3.670	--
1,2,3,6,7,9-	3.039	5.18	2.442	0.63	3.874	4.29
1,2,3,6,8,9-	3.376	3.66	2.488	0.51	4.276	--
1,2,3,7,8,9-	4.016	2.79	2.748	0.33	5.112	2.64
1,2,4,6,7,8-	2.434	2.72	2.101	0.22	3.108	2.82
1,2,4,6,7,9-	2.603	4.95	2.174	0.59	3.295	--
1,2,4,6,8,9-	2.893	3.69	2.217	0.53	3.631	--
1,3,4,6,7,8-	2.355	5.57	2.099	0.60	3.037	--
1,3,4,6,7,9-	2.381	2.76	2.140	0.34	3.025	2.88
2,3,4,6,7,8-	4.499	5.06	2.500	0.46	5.996	--
1,2,3,4,6,7,8-	4.025	--	3.333	2.61	5.708	9.35
1,2,3,4,6,7,9-	4.699	--	3.440	0.61	5.917	2.50
1,2,3,4,6,8,9-	4.699	--	3.507	1.35	6.501	4.58
1,2,3,4,7,8,9-	5.640	--	4.002	1.32	7.849	4.98
1,2,3,4,6,7,8,9-	7.809	--	5.708	0.43	11.854	1.68

[a] Source: Bell and Gara, 1985

[b] Retention times and response factors all given as ratios to those for 1,3,6,8-TCDF.

[c] Columns: SP-2330 fused silica 60 m x 0.25 mm ID at 240°C; DB-5 bonded phase fused silica, 60 m x 0.25 mm ID at 240°C; SP-2340 bonded fused silica 50 m at 220°C. The ECD was a ^{63}Ni-electron capture detector. The FID was a H_2/air flame ionization detector. The nitrogen carrier flow rate was 1-2 mℓ/min.

10 mℓ of n-hexane. PCDFs were recovered by elution with 6 mℓ of methylene chloride; the eluate evaporated and was subjected again to cleanup on a second identical aluminum oxide microcolumn. To achieve optimal removal of PCBs, 10 mℓ of 4% methylene dichloride/n-hexane was then used as the first eluent; PCDFs, lower PCBs, chlorinated tricyclic aromatics were then eluted with 10 mℓ of 50% methylene dichloride/n-hexane. This final eluate was concentrated and injected into the GC/MS. Recoveries of both 2,3,7,8-TCDF and TCDD were 90% at the 0.1-1 ppm level (Buser et al., 1978d). This is the basis of U.S. EPA method 613 except that 3% methylene dichloride/n-hexane and 20% methylene dichloride/n-hexane are used for the first and second eluents, respectively. RCRA Method 8280 also utilizes this step. Either basic or neutral alumina can be used with consistent results being obtained with a 3% methylene dichloride/hexane wash (10 mℓ), a 20% methylene dichloride/hexane wash (15 mℓ) with elution of PCDDs and PCDFs with 50% methylene dichloride/hexane (15 mℓ). If further cleanup is necessary a charcoal cleanup is recommended (Smith et al., 1984).

3.3.2.4.2. Yusho Oil -- Yusho oil (100 mg) was applied to a silica microcolumn (0.5 g silica gel, 70/230 mesh, Merck, in a 15 cm x 5 mm disposable Pasteur pipet). The PCBs and PCDFs were eluted with 6 mℓ of n-hexane with almost complete retention of rice oil on the column. The eluate was concentrated and applied to an aluminum oxide microcolumn (1.0 g basic aluminum oxide, Woelm, in a disposable Pasteur pipet, 15 cm x 5 mm). Most of the PCBs were eluted with 10 mℓ of 2% methylene dichloride/n-hexane and the PCDFs (plus other chlorinated tricyclic aromatics and lower chlorinated biphenyls) collected by elution with 10 mℓ of 50% methylene dichloride in n-hexane. The latter fraction was concentrated to 50 µℓ and 2 µℓ injected for GC/MS analysis. Recoveries of both 2,3,7,8-TCDF and -PCDD were

90% at the 0.1-1 ppm level (Buser et al., 1978d). This procedure differs slightly from the one quoted by Rappe et al. (1977) since the latter technique resulted in losses of some of the less polar PCDFs. This technique has also been used by Cull and Dobbs (1984), Rappe et al. (1985b) and Paasivirta et al. (1985).

PCB with soot or on wipes is generally treated with 1 M HCl for 1 hour by shaking, the slurry filtered by suction, washed with water, dried and then Soxhlet extracted with toluene and further treated as in Section 3.3.2.4.4. (Rappe et al., 1983a).

3.3.2.4.3. Chlorophenol Formulations -- A 4 g sample of the chlorophenol formulation was added to 30 mℓ of methanol in a 250 mℓ separatory funnel. Ten mℓ of 2.5 M lithium hydroxide was added and then 100 mℓ of water. The neutral fraction was extracted with 40 mℓ of light petroleum ether at pH 12. If the separation was difficult, 2-5 g of anhydrous sodium sulfate was added to break the emulsion. The organic phase was washed with 100 mℓ of 0.05 M lithium hydroxide, and then with 50 mℓ of distilled water, which should be neutral on separation. The organic phase was dried over anhydrous sodium sulfate and a 20 mℓ aliquot concentrated to 2 mℓ in a stream of nitrogen. The procedure for PCB formulations was followed, beginning at the second aluminum oxide column step, given above in Section 3.3.2.4.1. This method is a combination of the published methods of Buser and Bosshardt (1976) and Buser et al. (1978d). Recoveries of PCDFs were 90%. The method has been used also by Paasivirta et al. (1982) and Singh et al. (1985).

3.3.2.4.4. Fly Ash Samples -- Fly ash (25 g) was stirred in 1 M hydrochloric acid (200 mℓ). After centrifugation the residual fly ash was washed with deionized water in a Buchner funnel and dried at room temperature. The dried fly ash was then extracted for 35 hours with toluene using a Soxhlet apparatus. Approximately 98% was extracted in the first 24 hours, and in the next 24 hours 1.2-1.6% more was recovered (Kooke et al., 1981). This is the crucial step since other solvents like methylene dichloride, acetone/hexane or chloroform are much less efficient. Also, use of HF instead of HCl drastically lowers the extraction efficiency (Lustenhouwer et al., 1980; Kooke et al., 1981). Wang et al. (1983) showed that 12N HCl or 12N H_2SO_4 increases OCDD extraction by a factor of 2. The extract was then concentrated to 1 mℓ and loaded onto a chromatographic column consisting of 4 g of silica loaded with 40% (weight) concentrated sulfuric acid. This column was connected to another, containing layers of 2 g of sodium hydroxide treated silica gel (1 part by weight) of 1 M sodium hydroxide solution on 2 parts (by weight of silica gel), 2 g of silver nitrate on silica gel (10% by weight), and 1 g of silica gel. The columns were eluted with redistilled n-hexane. The eluent was concentrated and then chromatographed by the method described in Section 3.3.2.4.2. Recoveries of PCDDs and PCDFs were reported to be 100% (Lustenhouwer et al., 1980; Kooke et al., 1981). A quick method by Shushan et al. (1985) has not been adequately evaluated yet, but HPLC cleanup caused a large PCDF loss.

PCDF recovery from EPA-5 type sampling trains is extremely variable (Clement et al., 1985a; Wang et al., 1983; Tiernan et al., 1985); a multiple rinse scheme is necessary to achieve reasonable precision (Tiernan et al., 1985). An interlaboratory comparison is documented by Brocco et al. (1984), the results sometimes vary by an order of magnitude.

3.3.2.4.5. Agent Orange and 2,4,5-T Ester Formulations -- Ester formulation (1 g) was added in n-hexane to a silica gel column (15 cm x 11 mm; silica gel, Merck) and eluted with 30 mℓ of 5% methylene dichloride in n-hexane. The eluate was concentrated in a stream of nitrogen. The concentrate was subjected to the technique given in Section 3.3.2.4.1. starting at the second aluminum oxide column step. Recoveries of 20-50 μg of added TPCDDs and TCDFs were 90% (Rappe et al., 1978c).

3.3.2.4.6. Wood Dust -- A published method by Levin and Nilsson (1977) recommended that the wood dust be extracted with ether and the ether concentrate subjected to TLC or silica gel, and the PCDFs and PCDDs eluted with chloroform.

3.3.2.4.7. Gelatin Samples -- A 15 g gelatin sample was weighed into a 100 mℓ round bottom flask. Ethanol (20 mℓ) and 40% aqueous potassium hydroxide (40 mℓ) were added and the sample shaken for 1.5-2 hours at 180 strokes/minute. The solution was then extracted with hexane (1x20 mℓ; 3x18 mℓ) in a separatory funnel. The halogenated hexane extract was then extracted first with 1 M potassium hydroxide (40 mℓ) and then with 40 mℓ portions of concentrated sulfuric acid until the acid phase was colorless. Heavy emulsions at this last step were broken by swirling the separating funnel. Moderate emulsions were successfully broken by addition of a few crystals of anhydrous sodium carbonate. The washed hexane extract was further washed with water (10 mℓ) and saturated aqueous sodium carbonate (10 mℓ) and filtered through 5 cm (15 g) of anhydrous sodium carbonate in a 19 mm ID x 30 cm chromatographic tube. The hexane eluent was concentrated to 3 mℓ on a steam bath, the containing flask being filtered with a modified micro-Snyder column and also containing three 20-mesh carborundum boiling chips. Although Firestone (1977b) then utilized a slightly different method to achieve separation of PCDDs and PCDFs from other chlorinated compounds to obtain >90% recoveries of PCDDs (tetra- to hepta-) and 2,3,7,8- TCDF, the more modern procedure described in Section 3.3.2.4.4. should be followed before GC/MS.

3.3.2.4.8. Tissues -- Four methods are current: a Japanese method (Nagayama et al., 1977), one advocated by Albro and Corbett (1977), one by Stalling et al. (1981), and others compared by Albro et al., (1985).

The Japanese method, which was also used by Kuroki and Masuda (1977, 1978) and Rappe et al. (1979b), involves tissue homogenization in n-hexane and sodium sulfate in a Waring blender followed by saponification with 1 N potassium hydroxide in ethanol. The alkaline solution was extracted with n-hexane, concentrated and then chromatographed on a silica gel column (2 g) eluting with n-hexane. The concentrate was further fractionated on a column of aluminum oxide (3 g) using first n-hexane/carbon tetrachloride (4:1 v/v) and finally 25% methylene dichloride/n-hexane to elute the PCDFs and PCDDs. The final eluate was evaporated, so it could be injected into the GC. Albro and Corbett (1977) and Albro (1979) showed that there were extensive losses of OCDD and OCDF (up to 60%) at room temperature and 90% under reflux, during the potassium hydroxide saponification step. These losses are important if all PCDFs are to be quantitated or if these compounds take part in synergistic/antagonistic toxic reactions. The recovery of 2,3,7,8- TPCDD was 88-90% at room temperature.

Albro and Corbett (1977) described methods to extract PCDFs from liver, blood, urine, milk, serum and adipose tissues. Liver samples were blended in 200 mℓ of chloroform/methanol (2:1 v/v); the solution was filtered under suction through a glass fiber filter paper; and the filter paper and cake was then blended with 100 mℓ of chloroform/methanol (2:1 v/v). The solution was again filtered as above and the filtrates combined. A solution of 1.2% aqueous potassium chloride (52 mℓ) was added and mixed. The phases were allowed to clear, the lower phase collected, and evaporated just to dryness at 40°C by rotary evaporation under reduced pressure. Blood, urine, milk and serum (100 mℓ) were extracted as described by Kates (1972) and the resulting chloroform phase was concentrated by rotary evaporation. Adipose tissues were ground in a mortar and pestle with 8 times their weight of anhydrous sodium sulfate. The powder was extracted with chloroform in a Soxhlet, allowing at least eight cycles. The chloroform extracts were concentrated by rotary evaporation. All the chloroform concentrates obtained above were then further treated by the same cleanup procedure. The lipid residue from 10 g of liver, 100 mℓ of biological fluid or 1 g of adipose tissue was leached into 15 mℓ of carbon tetrachloride. Concentrated sulfuric acid (15 mℓ) was added, the mixture shaken and centrifuged at 2000 rpm for 30 minutes. The organic layer was removed, the volume noted and the layer dried by passing it through anhydrous sodium carbonate in a glasswool plugged funnel. The sodium carbonate was then rinsed with fresh CCl_4 (2 mℓ). The filtrate was concentrated just to dryness by rotary evaporation at 40°C. The residue was leached with 1.5 mℓ of n-hexane/methylene dichloride (97:3 v/v). The leachate was loaded onto a dry-packed column of Fisher A-540 aluminum oxide (3 g) of diameter 0.5-0.7 cm. The flask was rinsed with 1 mℓ of n-hexane:methylene dichloride (97:3 v/v) and added to the column. Fraction I was eluted with 28 mℓ of n-hexane:methylene dichloride (97:3 v/v). Fraction II was eluted with 30 mℓ of n-hexane: methylene dichloride (4:1 v/v). Fraction II was evaporated to near dryness. An aliquot was then taken for GC/MS analysis.

The recoveries of dibenzofuran, PCDFs and PCDDs, were reported to be 95% (using the method of standard additions). The sulfuric acid step was sufficient to handle 150 mg of triglyceride/mℓ of acid, but only about 50 mg of total liver lipid/mℓ of acid. Emulsions were routinely broken by centrifugation. Fisher A-540 aluminum oxide best retained lipids in the solvent system described; other aluminum oxide types allowed partial elution of sterol esters, waxy pigments and various triglycerides. The final concentrate was suitable for analysis at the ppt level by GC/MS.

The methods for PCDF quantitation in adipose tissues recommended by Stalling et al. (1981) [see also Rappe et al. (1981)] involved initial procedures similar to those described by Albro and Corbett (1977). Thus, adipose tissues were blended with 4 times their weight of anhydrous sodium sulfate and the column extracted with 100 mℓ volumes of methylene dichloride for every 10 g of tissue. After removal of solvent by rotary evaporation, the weight of the oil residue was noted. This residue was dissolved in 1:1 (v/v) cyclohexane/methylene dichloride to achieve an approximate concentration of 0.2 g/mℓ. Aliquots (5 mℓ) were applied to a gel permeation column containing 69 g SX-3 BioBeads resin (2.5 cm ID x 48 cm) and eluted at 5 mℓ/min. The 165-300 mℓ eluent fraction of each analysis was collected, amalgamated and then reduced to near dryness by rotary evaporation. The extract was then passed through a column of potassium hydroxide-treated silica gel topped with a small amount of cesium hydroxide-treated gel layered on top of a small band of sulfuric acid-impregnated silica gel. The eluent was then run immediately through a column containing carbon (Amoco PX-121; <40 μm) (Rappe et al., 1981) and dispersed on glass fibers (Stalling, et al. 1981). Planar molecules (such as, toxic PCDDS and PCDFS) were collected by reverse elution with toluene (30 mℓ), whereas nonplanar molecules could be eluted with 1:1 (v/v) benzene/ethyl acetate (30 mℓ) in the usual manner.

PCDDs and PCDFs were then specifically collected by the technique described in Section 3.3.2.4.2. before GC/MS. The reported recoveries were

>70%. A slightly revised method was used by Nygren et al., (1986) for human adipose tissue, mother's milk, bovine fat and bovine milk. The recovery of $^{13}C_{12}$-labeled surrogates is normally found to be >60%.

Albro et al. (1985) evaluated 6 different approaches in an interlaboratory study for 3 PCDFs (2,3,7,8-TCDF; 2,3,4,7,8-PeCDF; 1,2,3,7,8,9-HxCDF) added to human adipose tissue extracts at levels of 5 to 50 ppt. The adipose tissue (600g) was combined with 4.8 kg of pesticide grade anhydrous sodium sulfate in a clean food-grinder. After 3 processings, the powder was packed into two 20 in. x 4 in. diameter pyrex columns. The packing was eluted with 3ℓ of ethyl acetate containing 0.001% butylated hydroxytoluene, and then 6ℓ of methylene dichloride. The extracts were combined, concentrated in vacuo at 37°C, and the lipid diluted with chloroform to 900 mℓ to stop solidification. The yield was equivalent to soxhlet extraction with chloroform for 16 hours. The PCDFs in toluene were added and the adipose tissue solution (60g adipose tissue equivalent) stirred magnetically. The chloroform was reduced (water aspirator) in a dissicator over 24 hours. Six extraction methods were then evaluated.

(I) ^{13}C-2,3,7,8-TCDF and ^{37}Cl-2,3,7,8-TCDF were added as internal standards. The sample was passed in cyclohexane/CH_2Cl_2 through two potassium silicate/silica gel columns and then through carbon adsorbent (Stalling et al., 1981). The extracts were concentrated at 55°C in vacuo and then passed through a H_2SO_4/silica gel column in o-xylene. The interference of chlorinated naphthalenes can be further removed by a final alumina chromatography step. This method is derived from Smith et al. (1984).

(II) ^{13}C-2,3,7,8-TCDF was added as internal standard and the lipid solution partitioned between hexane/concentrated H_2SO_4 for 13-54 hours.

The organic phase was washed through silica, NaOH on silica, silica, H_2SO_4 on silica, and silica, with 5% benzene in hexane. After concentration, the residue was dissolved in hexane. This solution was then passed through $AgNO_3$ on silica and trapped on basic alumina. After washing with CCl_4/hexane (4:1), PCDF/PCDDs were eluted with CH_2Cl_2/hexane (3:1), the eluate evaporated under nitrogen and redissolved in chloroform. After reverse phase HPLC, the appropriate collected fractions were further purified by normal phase HPLC. This method is derived from Lamparski and Nestrick (1980).

(III) The ^{37}Cl-TCDF, PCDF, and HxCDF were added as internal standards. The samples were digested in concentrated HCl for 1 hour, extracted twice with hexane and dried over anhydrous sodium sulfate. The solution was washed through a H_2SO_4/silica gel column with hexane; the eluate was washed with water, dried and concentrated. The concentrate was bonded onto grade I alumina and washed with 5% CH_2Cl_2/hexane; the PCDFs/PCDDs were eluted with CH_2Cl_2/hexane (1:1), concentrated to 1 mℓ, and then loaded on Carbopack C/Celite treated as per Stalling et al. (1981). The extracts were concentrated to 10 µL with dodecane entrainer. This was an original method.

(IV) ^{37}Cl-2,3,7,8-TCDF was added as internal standard. The solution was then partitioned between hexane/H_2SO_4 until the H_2SO_4 was colorless. The hexane phase was washed with 1% aqueous NaOH and water, dried over Na_2SO_4/hexane, and eluted with CH_2Cl_2. The eluate was dried under nitrogen, and the residue redissolved in toluene or isooctane. This is a method derived by Ryan et al. (1985a).

(V) ^{37}Cl-2,3,7,8-TCDF was added as an internal standard. The solution was diluted and partitioned against H_2SO_4 and neutralized through

Ha_2CO_3 and KOH/silica. After concentration, the PCDFs were exchanged into hexane from cyclohexane, loaded onto acidic alumina, washed with 3% CH_2Cl_2/hexane, and eluted with CH_2Cl_2/hexane (1:1) onto charcoal/celite. This was then washed with 10% benzene/hexane and back-eluted with 50% xylene/hexane onto neutral alumina, washed with 3% CH_2Cl_2/hexane, eluted with CH_2Cl_2/hexane (1:1) and then concentrated. This is an original method.

(VI) ^{13}C-2,3,7,8-TCDF was added as internal standard. After dilution with CH_2Cl_2/cyclohexane, the solution was passed through silica/potassium silicate/Na_2SO_4/potassium silicate/silica/carbon column combinations. The last column was washed with cyclohexane CH_2Cl_2 and CH_2Cl_2/methanol/benzene (15:4:1), and back-eluted with toluene. The extracts were concentrated at 35°C, passed through potassium silicate/H_2SO_4 on silica with hexane onto acidic alumina, washed with hexane and 2% CH_2Cl_2/hexane. PCDFs/PCDDs were eluted with CH_2Cl_2/cyclohexane (1:1) and concentrated. This method was derived by Rappe et al. (1984). The molecular ions of all $^{13}C/^{35}Cl$ and $^{12}C/^{37}Cl$ PCDFs were monitored except in Method IV where the $(M - COCl)^+$ ion was monitored by MS/MS. The interferences from chlorinated diphenyl ethers, PCBs, chlorinated methoxy biphenyls, p,p'-DDE and o,p'-DDE were accounted for. Various GC columns were used with DB-5 and SP-23SO fused silica capillary columns were most utilized. Total analysis/report writing time varied between 48 and 140 days, though each method took between 6 and 26 working days.

The isotopic internal standard method was definitely superior to the external standard method (Table 3-7); the absolute quantitation is extremely variable. Only methods II, III and VI had relative standard deviations for unspiked samples of lower than 25%. As Table 3-7 shows, method II was most

TABLE 3-7

Error in Estimation of PCDFs by Six Methods[a]

Compound	% Relative Error Compared With "Correct" Level In Method					
	I	II	III	IV	V	VI
2,3,7,8-TCDD[b]	-50	+10	leaked	-90	-73	+70
2,3,7,8-TCDF[c]	-(1±3)	-(1±0)	+(2±3)	(0.5±11)	+(10±8)	+(3.5±2.1)
2,3,4,7,8-PeCDF[d]	+(13±6)	+(5±1)	+(12±7)	-(3.5±3.5)	+(27±16)	+(24±34)
1,2,3,7,8,9-HxCDF[e]	-73	-18	BD	-100	NM	+9

[a] Source: Albro et al. (1985)

[b] 10 ppt: (1 sample)

[c] 11 ppt: (average of 2 samples)

[d] 16 ppt: (average of 2 samples)

[e] 11 ppt: (1 sample)

BD = Below detection

NM = Not measured

accurate (within 20% of the correct answer) for spiked PCDF and 2,3,7,8-TCDD at ~10-16 ppt. This method took 26 working days for sample processing. There appeared to be no correlation between recoveries of PCDFs and PCDDs.

The results of this blind study included certain commonly encountered environmental contaminants that can interfere with the quantitation of PCDDs and PCDFs. Six laboratories using 6 different methods (all entailing GC-MS for quantitation of PCDFs) participated in the study. Comparison of the results indicated the data obtained were qualitatively reliable; however quantitation was problematical for some laboratories.

Another published report (Schecter et al., 1985a) describes the results of analyzing blood and adipose tissues from patients exposed to residues from a PCB-containing transformer fire. The method of analysis implemented in the latter study entailed GC-MS quantitation of not only 2,3,7,8-TCDD and 2,3,7,8-TCDF but the sum of the congeners constituting each chlorinated class of PCDDs and PCDFs, including the tetrachlorinated through octachlorinated congeners. The results of this study indicate that the tissue from patients with no known exposure to PCDDs or PCDFs had, in several cases, several hundred parts-per-trillion levels of higher chlorinated PCDDs and PCDFs. Other publications by Schecter et al. (1986a,b) and Ryan et al. (1986) have included the above approach.

3.3.2.4.9. Water -- There is currently no specific method for the analysis of PCDFs in water. The procedure used for urine in Section 3.3.2.4.8. but scaled up to accommodate 1 ℓ of water in the initial extraction stage should be sufficient. A simpler method would be to extract 1 ℓ (or a larger volume) of acidified water (pH 2) with methylene dichloride and then concentrate the extracts. The Yusho oil method (Section 3.3.2.4.2.) could then be applied. Obviously, further research is required in this area.

3.3.2.4.10. Sediment -- Sediment samples (10 g) have been analyzed by mixing and blending with 20 g anhydrous sodium sulfate, followed by Soxhlet extraction for 48 hours with 100 mℓ of toluene with subsequent concentration of the extract, before cleanup and GC/MS analysis (Petty et al., 1983a,b). The extraction efficiency is >74%. An article describing analytical methodology and the application of this method to the assessment of environmental contamination by PCDDs and PCDFs as a result of improper disposal of chemical wastes has been published (Tiernan et al., 1985). The analytical methodology employed entailed the use of GC-MS to quantitate parts-per-trillion to parts-per-billion levels of PCDFs as well as PCDDs in municipal refuse-fired boiler effluents and effluents from an RDF-fired boiler as well as soot from a PCB-containing transformer fire.

3.4. GC/MS METHODS FOR PBDFs

GC methods for the PBDFs are not as plentiful as those for PCDFs. O'Keefe (1978b) has published a method for separating PBDFs from PBBs. The PBB (50 mg) was dissolved in 2 mℓ of benzene and the solution was filtered through a sodium carbonate column (40 m x 6 mm) in a disposable Pasteur pipette, followed by two 5 mℓ benzene washings. The benzene eluate was concentrated to 1 mℓ, 10 mℓ of cyclohexane added, and the solution evaporated to 1 mℓ. This replacement procedure with cyclohexane was repeated twice. Methylene dichloride (15 mℓ) was then added, and the solution loaded onto a column of graphitized charcoal (Carbopack AHT; Supelco, Inc; 40 m x 6 mm).

Nonplanar aromatics were eluted with 50 mℓ benzene/diethyl ether (4:6) followed by pyridine (70 mℓ) to elute the planar aromatics. The pyridine fraction was reacted with 140 mℓ of 1% hydrochloric acid and then extracted with hexane (3x30 mℓ). The hexane fractions were combined, washed with

100 mℓ water, dried (sodium sulfate) and then concentrated. The concentrate was placed onto an activated aluminum oxide column (Woelm Neutral; 40x6 mm) in a disposable Pasteur pipet. The first eluate, 10 mℓ CCl_4, contained PBBs, and the PBDFs were eluted with 7 mℓ methylene dichloride. GC/MS was performed on this eluate. O'Keefe (1978b) used a 6 ft x 1/8 inch Pyrex column, packed with 3% OV-1 on 100/200 mesh Gas Chrom Q temperature programmed from 260-290°C at 6°C/min to separate any PBDFs before analysis by MS. The molecular weight of the TBDF was 479.6996, and it was the 100% peak (M). Other peaks were M-2Br (30%), M-COBr-2Br (20%), M-COBr (6%), M-Br (6%), M^{++} (3%) and $(M\text{-}2Br)^{2+}$ (weak). PeBDFs showed peaks at the molecular ion 561.606. The other major ions were M-COBr, M-Br, M-2Br and M-COBr-Br ions. This procedure allowed 50% recovery of the 2,3,7,8-TBDF and was applied to analyze pyrolyzed PBB.

No PBDFs (<0.5 ppm) were found in Firemaster BP-6 by Hass et al. (1978) using a technique involving Florisil fractionation of a 10 g sample dissolved in 150 mℓ of 3% methylene dichloride in hexane eluted first with 500 mℓ of 3% methylene dichloride/hexane and then with 500 mℓ of 50% methylene dichloride/hexane. The latter fraction contained 0.5 ppm PBDFs on concentration and analysis by GC/MS, using a 1 m x 2 mm ID stainless steel column packed with 3% OV-101 on 100/120 mesh Gas Chrom Q at 300°C. No recoveries of PBDF standards were cited and analytical conditions were not optimal with the use of the stainless steel GC column and the high temperature. A methyl-BDF was subsequently found in the polar fraction of a Firemaster of unspecified type (Moore, 1977).

Buser (1986) used a 25 m x 0.31 mm ID SE-54 glass capillary column (splitless) in conjunction with temperature programming. As expected, the retention times are longer and the elution temperatures are higher than the corresponding PCDF analogs. The electron impact mass spectra of TBDF and PeBDFs agreed with those found by O'Keefe (1978b).

3.5. OTHER METHODS FOR THE PCDFs

It is clear from Section 3.3. that no one technique will suffice to separate, quantify and confirm the presence of the PHDFs. Sometimes it is necessary to use all the available methods.

Hutzinger et al. (1973) found that TLC on Merck Silica Gel F-254 of 0.25 mm thickness allowed 2,8-DCDF and OCDF to be separated in n-hexane solvent with R_f values of 0.55 and 0.75, respectively.

A gel permeation technique for the cleanup of PCDDs and PCDFs was proposed by Stalling et al. (1975). The system used BioBeads S-X_3 with ethyl acetate and ethyl acetate/toluene as solvents. This system has been alluded to in Section 3.3.2.4.8. PCDFs, PCDDs and chloronaphthalenes were retained on a charcoal column subsequent to gel permeation chromatography and were eluted with ethyl acetate/toluene.

Perchlorination techniques have been used to obtain total PCDF content (Masuda et al., 1976), but for reasons outlined previously are not useful in identifying individual toxic isomers. Ballschmiter et al. (1985) have used BMC reagent to achieve perchlorination. Perbromination is not possible because of steric hindrance (Richtzenhain and Schrage, 1978).

The Firemaster BP-6 metabolite, 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl decomposes on OV-101 GC columns at 230-260°C to form two PeBDFs (Gardner et al., 1979). This demonstrates that PBDF detection alone does not prove PBDFs were originally present in the sample. If phenolic metabolites are separated, there is no lack of specificity. There is also the need for a good HPLC method along the lines of the separation of PCDD isomers (Nestrick et al., 1979). The latter step should complement the GC step of GC/MS. Substantial HPLC losses especially for lower PCDFs have been reported (Shushan et al., 1985). The losses vary from 2- to 5-fold. Clearly this warrants investigation. Mazer et al. (1983a,b) have successfully used a reverse phase column (C_{18}) with 75% acetonitrile/25% water at 0.75 mℓ/min with detection at 235 nm. They also utilized a normal phase Zorbax NH_2 column with hexane as the eluent at 1.5 mℓ/min. Both columns were used in cleanup steps to produce pure isomers rather than to provide quantitative recovery. Cull and Dobbs (1984) have also used this technique. The quality control and assurance required for HPLC analysis of PCDDs and PCDFs has been presented by Donnelly et al. (1986) in connection with RCRA Method 8280.

A promising radioimmunoassay for 2,3,7,8-tetra-PCDF in PCBs and in environmental samples has been recently developed (Luster et al., 1980) as a screening technique.

Biological monitoring by induction of arylhydrocarbon hydroxylase activity (Poland et al., 1979; Bradlaw and Casterline, 1979) is considered in Chapter 7. Hutzinger et al. (1981) and Wang et al. (1983) showed that the toxicity of fly ash samples in the cytosol receptor assay could not solely be explained in terms of 2,3,7,8-TCDD (72 pg 2,3,7,8-TCDD equivalents was the ED_{50}). The induction and cytosol receptor assay tests often disagreed (Sawyer et al., 1983). See Chapter 7 for a discussion of these tests.

Helder et al. (1982) and Helder and Seinen (1985) have used a biological assay with rainbow trout yolk sac fry to show that the toxicity of fly ash cannot be solely attributed to the 2,3,7,8-TCDD level but to the cumulative presence of other PCDDs and PCDFs. The fry suffered hemorrhages, edema, fin necrosis and finally death at days 4-12. 2,3,7,8-TCDF also caused this behavior. The raw fly ash was as toxic as the cleaned-up PCDDs/PCDFs from the same fly ash sample reconstituted in the same volume.

A bioassay based on humoral antibody production has been utilized by Rizzardini et al. (1983). 2,3,7,8-TCDF at 10 ng/g caused an immunosuppression of 25%. Administration of both 2,3,7,8-TCDD and 2,3,7,8-TCDF caused much less immunosuppression than the TCDD alone.

4. SOURCES TO THE ENVIRONMENT

4.1. SUMMARY

PCDFs occur as contaminants of a number of chemical products such as phenoxy herbicides, polychlorinated phenols, hexachlorobenzene, polychlorinated biphenyls and chlorodiphenyl ethers. PCDFs are also associated with thermal insulation materials. Thus, both PCDFs and PBDFs are adventitious copollutants released into the environment together with economic poisons or industrial wastes. There is experimental evidence that PCDFs and PBDFs may be formed in the environment by photolytic and pyrolytic reactions from chemical products and combustion processes. Many of the products contain a larger percentage of PCDDs than PCDFs. However, the PCDFs in these products include more of the tetra- and penta- (the most acutely toxic) compounds than do the PCDDs. The levels of PCDFs containing 4 and 5 halogen atoms are generally <20 ppm for most chlorophenols, <0.40 ppm in most phenoxy herbicides, <5 ppm in PCBs and <0.5 ppm in the PBB, Firemaster BP-6. The lower chlorinated phenols and PCBs are most highly contaminated with toxic PCDFs. The levels of the 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, the most toxic isomers, are generally <1 ppm in most PCBs.

PHDFs are formed by the pyrolysis of various materials. They have been detected in incinerator fly ash ($\leq$1.5 ppm), in pyrolyzed PCB (up to 10^4 ppm), in PCB that had been used for 2 years as heat exchanger fluid ($\leq$16 ppm), in pyrolyzed PBB (up to 10^4 ppm), in pyrolyzed (2-butoxyethanol) ester of 2,4,5-T (<20 ppm), in some pyrolyzed commercial chlorophenols and in pyrolyzed chlorinated diphenyl ethers.

4.2. SOURCES OF PCDFs FROM CHEMICALS

The early work on the detection of PCDFs was hampered by losses during sample cleanup and analysis and by the fact that many PCDF gas chromatographic peaks were frequently superimposed on those of chlorinated diphenyl ethers (Crummett and Stehl, 1973). The interfering chlorinated diphenyl ether usually contained two chlorine atoms more than the PCDF. In spite of these difficulties, OCDF and a HpCDF were detected in dipping wastes containing pentachlorophenolate used as a wood preservative attributable to side-reactions in manufacture (Jensen and Renberg, 1973).

PCDFs are therefore not commercially produced but are formed as trace unwanted impurities in the manufacture of other chemicals such as chlorinated phenols and their derivatives, chlorinated diphenyl ethers, hexachlorobenzene and polychlorinated biphenyls, which are released into the environment either as economic poisons or as industrial wastes (Table 4-1).

An estimate of the extent of release into the Canadian environment of known sources of PCDFs is provided in Table 4-2 (Sheffield, 1985a,b).

4.2.1. Chlorinated Phenols. Chlorophenols are industrially produced by two methods, namely by the direct chlorination of phenol (Hutzinger et al., 1985b) and by alkaline hydrolysis of chlorobenzene in different solvents (Ahlborg and Thunberg, 1980). PCDFs appear as contaminants in both processes with more formed in the latter process. The annual world production in 1979 was ~150,000 tons (Rappe et al., 1979a).

Chlorinated phenols are used as fungicides, herbicides, slimicides, wood preservatives and bactericides; they are also used in the synthesis of chlorinated phenoxy herbicides.

Table 4-3 provides the composition of two typical commercial PCPs. Table 4-4 summarizes some of the PCDF and PCDD levels found in various chlorinated phenols. As expected, the higher PCDDs and PCDFs predominated with the PCDDs usually more abundant. However, the TCDFs and PeCDFs in all chlorophenols were greater than the content of the corresponding PCDD congeners. Since the HpCDDs, HpCDFs, OCDDs and OCDFs have lower toxicity

TABLE 4-1

Presence of PCDFs in Industrial Chemicals and Their Entry into the Environment

Industrial Products Containing PCDFs	Use	PCDFs (total concentrations in industrial products)*	
		Concentration in mg/kg (min/max)	Reference
Phenoxy alkanoic compounds	herbicides	0.008-0.15	Rappe et al., 1978b, 1979a; Ahling et al., 1977
Chlorinated phenols	wood preservative	59.8-790	Rappe et al., 1979
Hexachlorobenzene	fungicide and industrial waste	0.35-58.3	Villaneuva et al., 1974
Polychlorinated biphenyls	industrial use	0.8-13.6	CNRC, 1978b
Chlorides of iron, aluminum and copper	industrial use	0.0003-0.060	Heindl and Hutzinger, 1986

*Only positive data are considered

TABLE 4-2

Estimated PCDD and PCDF Release to the Canadian Environment from Chemical Sources*

Source	Release (g/year)	
	PCDD	PCDF
Chlorophenol production		
Air emission	900	600
Wastewater	13	13
Processes using pentachlorophenol		
Wood preservation		
Wastewater	1000	600
Waste disposal	>500	>300
Leather tanning		
Sludge	negligible	
Chemical products		
2,4-D formulations	100	
2,4,5-T	5	
Pentachlorophenol	[1.1×10^6]	[0.6×10^6]
PCBs		[750,000]

*Source: Sheffield, 1985a,b

TABLE 4-3

Chemical Analysis of a Typical Pure and Technical Pentachlorophenol[a]

Source	Pure (Aldrich, lot 120,717)	Technical (Monsanto, lot KA578)
Phenols[b]		
Pentachlorophenol	>99%	84.6%
Tetrachlorophenol	<0.1 ppm	3%
Nonphenolics (ppm)[b]		
Dibenzo-p-dioxins		
Tetrachloro-	<0.1 ppm	<0.1 ppm
Pentachloro-	<0.1	<0.1
Hexachloro-	<0.1	8
Heptachloro-	≤0.1	520
Octachloro-	≤0.1	1380
Dibenzofurans		
Tetrachloro-	<0.1	≤4
Pentachloro-	<0.1	40
Hexachloro-	<0.1	90
Heptachloro-	<0.1	400
Octachloro-	<0.1	260

[a] Source: Goldstein et al., 1977a

[b] Samples were analyzed by GC/MS. The lower detection limit was 0.1 ppm.

TABLE 4-4

Levels of PCDFs and PCDDs in Some Commercial Chlorophenols

Chlorophenol	Formulation Type	Levels of PCDFs and PCDDs (mg/kg)												Reference
		Tetra-		Penta-		Hexa-		Hepta-		Octa-		Total		
		CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	
Penta-	(Monsanto)	<4	<0.1	40	<0.1	90	8	400	520	260	1380	794	1908	Goldstein et al., 1977a
Penta-	(Dow)	0.20	<0.02	0.20	<0.02	13	10	70	130	55	210	138	350	Rappe et al., 1979a
Penta-	(Dowicide 7)	-	-	-	-	30	4	80	125	80	2500	190	2629	Firestone, 1977a
Penta-	(Dowicide 7)	<0.2	<0.2	<0.2	<0.2	39	9	280	235	230	250	549	494	Buser, 1975
Penta-	(Dowicide EC-7)	-	-	-	-	<1	1	1.8	6.5	<1	15	3.8	22.5	Firestone, 1977a
Penta-	(Dowicide EC-7)	0.45	-	0.03	-	0.30	0.15	0.50	1.1	0.5	5.5	1.8	6.8	Buser and Bosshardt, 1976
Penta-	(Dowicide EC-7)	<0.02	-	<0.03	-	<0.03	0.03	0.5	1.1	0.2	5.5	<0.08	6.6	Buser and Bosshardt, 1976
Penta-	(Dow)	0.20	-	0.20	-	13	10	70	130	55	210	135	350	Buser and Bosshardt, 1976
Penta-	(Dow)	0.07	-	0.20	-	9	5.4	60	130	65	370	134	3.5	Firestone, 1977a
Penta-	(Monsanto)	-	-	-	-	19	11	81	199	137	1170	237	1300	Firestone, 1977a
Penta-	(Monsanto)	-	-	-	-	90	8	400	520	260	1380	750	1980	Firestone, 1977a
Penta-	(European)	0.9	?	4	?	32	?	120	?	130	?	280	1000	Rappe et al., 1978a,b

TABLE 4-4 (cont.)

Chlorophenol	Formulation Type	Levels of PCDFs and PCDDs (mg/kg) Tetra- CDF	Tetra- CDD	Penta- CDF	Penta- CDD	Hexa- CDF	Hexa- CDD	Hepta- CDF	Hepta- CDD	Octa- CDF	Octa- CDD	Total CDF	Total CDD	Reference
Penta-	(Fluka)	<0.21	-	0.05	?	15	9.5	95	125	105	160	220	295	Buser and Bosshardt, 1976
Penta-	(Fluka)	0.05	-	0.25	-	36	9.1	320	180	210	280	566	469	Buser and Bosshardt, 1976
Penta-	Na Salt (Fluka)	<0.02	0.12	0.03	0.03	0.7	<0.03	1.3	0.3	2.1	1.5	4.23	2.0	Rappe et al., 1978a,b
Penta-	Na Salt (Dow)	<0.02	-	0.005	-	11	3.4	50	40	24	115	85	158	Rappe et al., 1978a,b
Penta-	Na Salt (Fluka)	<0.02	0.05	0.04	<0.03	11	3.4	47	38	26	110	84	151	Buser, 1976a
Penta-	K_{12}	ND	ND	5.9	92	3.0	1921	29	204	11	-	-	-	Paasiverta et al., 1982
Penta-	K_1	ND	ND	ND	ND	98	3.5	39	1.1	1.5	-	-	-	Paasiverta et al., 1982
Penta-	8 samples	-	-	-	-	-	4.5	62	35	130	610	-	-	Cull and Dobbs, 1984
Tetra- (2,3,4,6-)	(Dowicide 6)	<0.2	<0.2	<0.2	<0.2	30	6	500	55	135	39	865	100	Buser, 1975
Tetra- (2,3,4,6-)	(Finnish) Kymmene Ky-5	<0.5	<0.7	10	5.2	70	9.5	70	5.6	10	0.7	160	22	Rappe et al., 1978a,b
Tetra-	Ky-5	2.0	2.1	2.8	-	21	-	30	0.6	4.0	0.8	-	-	Paasiverta et al., 1982
Tri-	(Swedish)	1.5	<0.02	18	<0.03	36	<0.03	4.8	<0.1	-	<0.1	60	<0.3	Rappe et al., 1977

(McConnell et al., 1978b; Poland and Glover, 1977), it may seem more appropriate to concentrate on the TCDFs to HxCDFs. This can be misleading, however, since 2,3,7,8-TCDD is 1000-10,000 times more toxic than 1,2,3,8-TCDD (McConnell et al., 1978b; Poland and Glover, 1977). Nevertheless, the probability of encountering a toxic compound may be much greater for PCDFs than for a PCDD since there are more PCDFs containing 4 or 5 chlorine atoms in the vicinal positions, where toxicity is conferred. Firestone (1977a) reported that the major PCDFs in Dowicide 7 and EC-7 formulations were the 2,3,6,7- and 2,4,6,7-tetra-; the 2,3,4,6,7-, 1,2,4,7,8-, 2,3,4,7,8-penta-; and the 1,2,3,6,7,8-hexa-isomers. Rappe et al. (1978a,b) found the major components in an American PCP to be those given in Table 4-5. The isomers present were very different from those reported by Firestone (1977a). As shown in Table 4-5, the European trichlorophenol formulation contained traces of the toxic 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF.

In the United States, PCP is manufactured by the chlorination of phenol (Firestone, 1977a). In Europe, both this process and the alkaline hydrolysis of hexachlorobenzene (135-275°C) have been used. The product from chlorination of phenol tends to have comparatively nontoxic, higher chlorinated isomers. This process is conducted at atmospheric pressure using two reactors. In the first, phenol is chlorinated at 65-130°C until 3-4 chlorines have been added and until the mp of the product has reached 95°C. The temperature is then adjusted slowly into the 195-205°C range, a process typically complete in 5-15 hours. Aluminum chloride is often added to accelerate the final chlorination. Typically, the final product consists of 80-88% PCP and 12-20% 2,3,4,6-tetrachlorophenol in addition to hydroxypolychlorinated diphenyl ethers, PCDDs, PCDFs, chlorodioxins (1000-3000 ppm),

TABLE 4-5

Order of Abundance of PCDFs in European Chlorophenols[a]

2,4,6,-Trichlorophenol	2,3,4,6-Tetrachlorophenol	Pentachlorophenol
1,2,3,4,6,8-Hexa-[b]	1,2,3,4,6,8-Hexa-[b]	1,2,3,4,6,8,9-Hepta-[b]
1,2,4,6,8-Penta-[b]	1,2,4,6,8,9-Hexa-[b]	1,2,4,6,8,9-Hexa-[b]
1,2,4,6,8,9-Hexa-[b]	1,2,3,4,6,7,8-Hepta-	1,2,3,4,6,7,8-Hepta-
1,2,4,6,7,8-Hexa-[b]	1,2,3,4,6,8,9-Hepta-	1,2,4,6,7,8-Hexa-
2,3,4,6,8-Penta-	1,2,4,6,7,8-Hexa-	1,2,4,6,8-Penta-
2,3,7,8-Tetra-[c]	1,2,4,6,8-Penta-	1,2,3,4,6,8-Hexa-
2,4,6,8-Tetra-	2,3,4,6,7,8-Hexa-(trace)	1,2,3,4,7,8,9-Hepta-(trace)
1,3,4,7,8-Penta-		Octa-
1,2,3,4,6,7,8-Hepta-		
2,3,4,7,8-Penta-		
1,2,3,4,6,8,9-Hepta-		
2,3,6,8-Tetra-		
1,2,3,7,8-Penta-		
2,3,4,6,7,8-Hexa-		

[a] Source: Rappe et al., 1978a,b

[b] Most abundant

[c] Subsequent work (Mazer et al., 1983a,b) revealed that 2,3,4,8-TCDF co-elutes.

chlorofurans (200-600 ppm) and chlorinated diphenyl ethers (Firestone, 1977a). Poor temperature control in this final phase has been postulated to account for the formation of PCDFs (and PCDDs) from the chlorinated diphenyl ethers (Firestone et al., 1973). The length of time that chlorination continues undoubtedly has some influence, since refluxing of PCP in benzene for 6 hours with chlorine bubbling can produce OCDF in 52% yield (Plimmer, 1973).

Commercial sodium 2,4,5-trichlorophenolate tends to contain more PCDDs and PCDFs than the chlorophenol itself (Firestone et al., 1972). Most of the PCDFs possessed 3-6 chlorine atoms. While 2,3,4,6-tetrachlorophenol formulations did contain TCDFs to OCDFs (see Table 4-4), no PCDFs or chloroethers were found in samples of 2,4- or 2,6-dichlorophenol.

Levin and Nilsson (1977) reported that Swedish sawmill workers were exposed to PCDFs (levels up to 6 ppm) in Finnish 2,3,4,6-tetrachlorophenolate in the sawdust.

PCP has been detected in urban air (at concentrations of 0.25-0.93 and 5.7-7.8 ng/m^3), in waters from various manufacturing and processing plants, in sewage-plant effluent (1-5 μg/ℓ) in rain, snow and lake waters (2284, 14 and 10 ng/ℓ), in creek water containing industrial wastes (at levels of 0.1-10 mg/ℓ); soil residues after application of 15 and 45 kg/ha PCP were 20.4 and 69.1 mg/kg (WHO, 1979). Trichlorophenol (unspecified isomers) has been identified in river water, tap water (2.4 mg/ℓ), landfill leachate (40 mg/ℓ), and in effluent from sewage treatment plants (WHO, 1979). Other contaminants such as PCDDs and polychlorinated diphenylethers have been identified in technical grade PCP (Rappe et al., 1978a, 1979a; Nilsson and Renberg, 1974; Buser, 1975).

4.2.2. PCDFs in Polychlorinated Biphenyls. PCBs are mixtures of chlorinated biphenyls widely used in a number of industrial applications, such as heat exchange, dielectric fluids, hydraulic and lubricating fluids, plasticizers, printing inks and flame retardants (WHO, 1976). Large amounts of PCBs have been released into the environment since 1929. Because of the stability of some isomers to chemical and biological attack, they are today ubiquitous and persistent pollutants of ecosystems (Wassermann et al., 1979).

The presence of PCDFs in PCBs has been detected by several authors in American, European and Japanese commercial PCB mixtures (Vos et al., 1970; Nagayama et al., 1976; Roach and Pomerantz, 1974; Bowes et al., 1975a). Because of production differences the PCDF concentrations and isomers vary with PCB type and origin. Bowes et al. (1975b) found levels of 0.8 and 2 ppm in American-made Aroclor 260 and 1248, respectively; 8.4 and 13.6 ppm of PCDF were estimated in German Clophen A-60 and French Phenoclor DP-6 (Nisbet, 1976). In the late 1960s, a significant difference in toxicity to chick embryos was discovered among three supposedly identical PCB formulations originating from three different countries (Vos and Koeman, 1970). Large amounts of comparatively more polar contaminants were detected in a German PCB (Clophen A-60) and a French PCB (Phenoclor DP-6) but not in an American PCB (Aroclor 1260). Two of the contaminants in the German PCB were identified by GC/MS as a TCDF and a PeCDF (Vos et al., 1970); these compounds apparently accounted for the observed acute toxicity of the whole PCB formulation. PCDFs were found also in American PCBs but not as much as in the European formulations (Bowes et al., 1973). Kanechlors from Japan were also found to contain PCDFs (Bowes et al., 1975a; Roach and Pomerantz, 1974). The average PCDF content in Aroclors 1248, 1254 and 1260 from the United States was then put between 1 and 2 ppm. Less than ppt levels were detected in Aroclor 1016 (Bowes et al., 1975a,b). The 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF were detected in Aroclors 1248 and 1254, and in Kanechlors

200 and 500 (Bowes et al., 1975a,b); Clophen A-60 contained ~8 ppm PCDFs, and Phenoclor DP-6 contained ~14 ppm. The PCBs with the highest PCDF contents were toxic to chick embryos.

In 1968, some 1200 Japanese people consumed a rice oil (Kanemi) contaminated with 800-1000 ppm of a Japanese PCB formulation, Kanechlor 400, which had leaked from a heat exchanger (Nagayama et al., 1975). This caused Yusho disease. Several months before this incident, 0.50 million chicks were killed by a crude bran oil from the same origin but the warning went unheeded (Kohanawa et al., 1969). The composition of Kanechlor 400 resembled that of Aroclor 1248. Of the GC peaks assigned to PCDFs six made up 2 ppm of the PCB. However, reports of the total PCDF content varied greatly from investigator to investigator. For example, Roach and Pomerantz (1974) detected 1 ppm, Nagayama et al. (1975) 18 ppm and Kuratsune et al. (1976) reported 33 ppm. These values were obtained on packed GC columns and so are expected to be maximal figures. Later capillary-GC work confirmed a level of 2-5 ppm (Nagayama et al., 1977; Rappe et al., 1977), the major PCDFs being the toxic 2,3,7,8-TCDF (0.45 ppm) and the 2,3,4,7,8-PeCDF (~0.2 ppm). It was calculated that the contaminating Kanechlor 400 PCB contained a PCDF level 250 times greater than the value found for an unused Kanechlor 400 formulation (Nagayama et al., 1975). A 4-fold increase of PCDFs (15-20 ppm) occurred when a PCB was used for 2 years in a heat exchanger in a situation similar to the suspected source of rice oil contamination (Morita et al., 1977a). More than 40 isomers were found; of those found, the 2,3,7,8-TCDF was the most abundant (1.25 ppm). Later work (Mazer et al., 1983a,b; Rappe et al., 1984) showed that the 2,3,4,8-TCDF co-eluted. The chromatographic profile of the TCDF fraction was very similar to that of Kanemi oil (Buser et al., 1978d; Rappe et al., 1977). A summary of the levels of PCDFs in various PCB formulations and in Kanemi oil is given in Table 4-6.

Table 4-7 gives the suspected maximum levels of the toxic 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF in these formulations (Morita et al., 1977a). The levels of the 2,3,7,8-derivatives were identified by retention time of the pure standard and were subsequently confirmed by Buser et al. (1978d) using the GC/MS. If the same order of elution is followed on the GC columns utilized by these workers, probably peaks 6-10 and 13-19 of Buser et al. (1978d) correspond to peaks 2 and 3, respectively, of Morita et al. (1977a) (Figure 4-1). Similarly, peaks 23-33, 37-40, 44-47, 51-52, 53-56, 58-66, 68-70, 71-73, 77-78 and 85 denoted by Buser et al. (1978d), correspond to peaks 3, 5, 6, 9, 10, 11, 12, 14, 16 and 17 of Morita et al. (1977a). The 2,3,7,8-TCDF-derivative, therefore, constitutes >90% of peak 6 as observed by Morita et al. (1977a), accounting for the near equivalence of the levels of the 2,3,7,8-TCDF found in Kanemi oil by both sets of workers. Later it was found that the 2,3,4,8-TCDF co-eluted (Mazer et al., 1983a,b; Rappe et al., 1984). The actual level of the 2,3,4,7,8-PeCDF is probably ~70% of the levels quoted in Table 4-7, since peak 68 of Buser et al. (1978d) is 70% of the combined height of peaks 68, 69 and 70 corresponding to peak 12 of Morita et al. (1977a). The figures quoted in Table 4-7 are therefore unlikely to have an error >30% provided that the elution profiles on the two GC columns are comparable. Thus, these two toxic isomers may have made up between 5 and 34% of the total PCDFs in the PCBs. The low values for the Aroclors T-1242 and T-1260 are to be noted. The low toxicity of Aroclor 1260 can thus be correlated with the low level of toxic CDBF (Vos and Koeman, 1970).

TABLE 4-6

PCDF Content in Some PCBs and in Kanemi Oil[a]

Substrate	PCDF Content (ppm)					Reference
	Tri-	Tetra-	Penta-	Hexa-	Total	
Yu-Cheng oil[b] (3)					0.14-0.18	Miyata et al., 1985
Kanemi oil[b] (4) (Yusho)					2-7	Miyata et al., 1985
Unused Kanechlor 400[b]					33	Miyata et al., 1985
Used Kanechlor 400[b] (3)					20-510	Miyata et al., 1985
Kanemi oil (Yusho)	0.15	1.4	2.5	1.6	5.7	Buser et al., 1978d
Kanemi oil (Yusho)	--	--	--	--	5.0	Nagayama et al., 1976
Used Japanese PCB (Mitsubishi-Monsanto T-1248)	4.2	4.5	5.5	1.4	16	Buser et al., 1978d
Kanemi oil	0.02	0.52	1.3	0.81	2.7	Morita et al., 1977a
Kanechlor 300	--	6.7	1.6	--	8.3	Morita et al., 1977a
Kanechlor 300	--	--	1.3	--	1.3	Nagayama et al., 1976
Kanechlor 400	0.3	12.2	10.4	0.9	24	Morita et al., 1977a
Kanechlor 400	--	--	--	--	18	Nagayama et al., 1976
Kanechlor 400	--	1	--	--	--	Roach and Pomerantz, 1974
Kanechlor 500	0.2	1.7	1.1	3.1	6.1	Morita et al., 1977a
Kanechlor 500	--	--	--	--	3.3	Nagayama et al., 1976
Kanechlor 600	--	0.2	0.5	0.4	1.1	Morita et al., 1977a
Kanechlor 600	--	--	--	--	4.0	Nagayama et al., 1976
Phenoclor DP-4	--	1.7	1.6	0.5	3.8	Morita et al., 1977a
Phenoclor DP-5	--	4.6	2.7	2.6	9.9	Morita et al., 1977a

TABLE 4-6 (cont.)

Substrate	PCDF Content (ppm)					Reference
	Tri-	Tetra-	Penta-	Hexa-	Total	
Phenoclor DP-6	0.2	2.1	2.6	5.6	11	Morita et al., 1977a
Phenoclor DP-6	--	0.7	10	2.9	14	Bowes et al., 1975a,b
Aroclor T-1200	--	0.1	0.4	0.5	1.0	Bowes et al., 1975a,b
Aroclor T-1241	--	2.4	2.7	0.8	5.9	Morita et al., 1977a
Aroclor T-1242	--	2.3	2.3	--	4.5	Morita et al., 1977a
Aroclor T-1248	--	0.5	2.3	--	2.8	Morita et al., 1977a
Aroclor T-1248[c]	0.3	5.8	5.6	0.7	12	Morita et al., 1977a
Aroclor T-1254	--	0.1	0.2	1.4	1.7	Bowes et al., 1975a,b
Aroclor T-1254	--	0.2	0.4	0.9	1.5	Bowes et al., 1975a,b
Aroclor T-1254	--	0.1	3.6	1.9	5.6	Morita et al., 1977a
Aroclor T-1260	--	0.2	0.3	0.3	0.8	Bowes et al., 1975a,b
Aroclor T-1260	--	0.8	0.9	0.5	2.2	Morita et al., 1977a
Aroclor T-1264	--	4.8	9.4	2.0	16	Morita et al., 1977a
Clophen A-30	1.6	2.3	1.0	--	4.9	Morita et al., 1977a
Clophen A-40	1.5	5.4	6.9	--	14	Morita et al., 1977a
Clophen A-50	0.7	8.3	4.1	1.8	15	Morita et al., 1977a
Clophen A-60	--	1.4	5.0	2.2	8.4	Bowes et al., 1975a,b

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[a] No data found for hepta-

[b] Yu-Cheng oil also contained 22-113 ppm PCBs, 9-38 ppm PCQs. Kanemi oil also contained 151-968 ppm PCBs, 490-866 ppm PCQs. Kanechlor 400 also contained 999,800 ppm PCBs, 209 ppm PCQs. Used Kanechlor 400 also contained 961,900-999,000 ppm PCBs, 690-31,000 ppm PCQs.

[c] Used PCB

TABLE 4-7

Suspected Maximum Levels of Toxic PCDFs in Various PCBs and in Kanemi Oil (Kanemi oil also contained 900 ppm PCBs and 800 ppm PCQs;[a] Taiwan rice oil also contained 60-100 ppm PCBs and 90-180 ppm PCQs)

	PCDF Levels (ppm)			
Formulation	2,3,7,8-[b,c] TCDF	2,3,4,7,8-[b,d] PeCDF	Total	Percentages of Total PCDFs for These Two Derivatives
Taiwan rice oil (2)[a]	0.001-0.005	0.02-0.70	0.08-0.10	20-25
Kanemi oil[a]	0.2	0.7	2.02	45
Kanemi oil[b]	0.28	0.42	2.68	26
Phenoclor[a]				
DP-4	0.7	0.4	3.8	29
DP-5	2.2	0.8	9.9	30
DP-6	0.9	0.6	10.5	14
Kanechlor[b]				
KC-300	2.2	0.6	8.3	34
KC-400	1.6	0.9	23.8	11
KC-500	0.7	0.7	6.1	23
KC-600	0.1	0.1	1.1	18
Aroclor[b]				
T-1241	1.1	0.4	5.9	25
T-1242	0.2	0.1	4.5	7
T-1248	0.2	0.8	2.8	36
T-1248[e]	1.1	1.4	12	20
T-1254	--	1.6	5.6	29
T-1260	--	0.1	2.2	5
T-1264	2.4	2.3	16	29

TABLE 4-7 (cont.)

Formulation	PCDF Levels (ppm) 2,3,7,8-[b,c] TCDF	PCDF Levels (ppm) 2,3,4,7,8-[b,d] PeCDF	Total	Percentages of Total PCDFs for These Two Derivatives
Clophen[b]				
A-30	1.0	0.1	4.9	22
A-40	2.1	0.7	14	20
A-50	3.6	0.6	15	28

[a] Masuda et al., 1982

[b] Calculated from Morita et al., 1977a

[c] Based on GC retention time, but subsequently confirmed by Buser et al., 1978d

[d] Assumed the order of elution obtained by Buser et al., 1978d, is followed on the GC column utilized

[e] Used PCB

-- Below detection limit

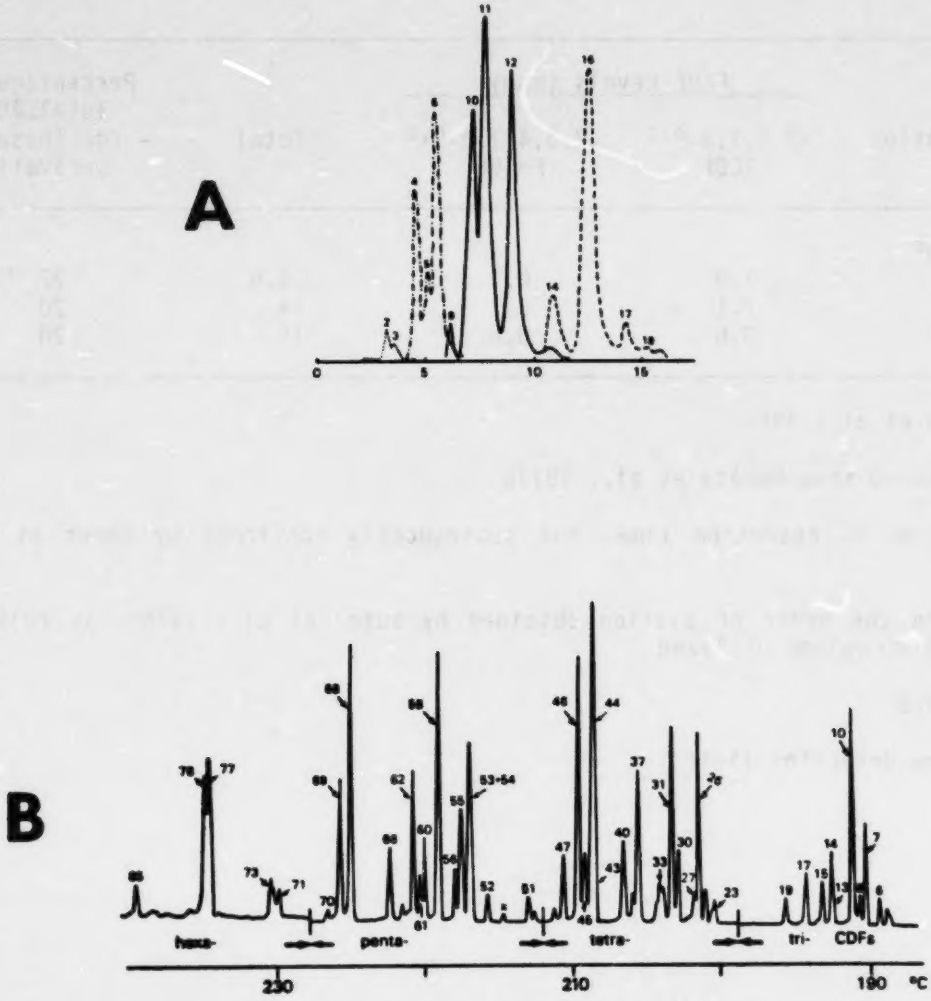

FIGURE 4-1

Comparison of Specific Ion Current Profiles after Chromatography of Kanemi Oil

Source: (A) Morita et al. (1977a); (B) Buser et al. (1978d)

A similar incident occurred in Taiwan in 1978 affecting nearly 2000 people. This has been dubbed the "Yu-Cheng" episode (Chen et al., 1981; Kashimoto et al., 1981). The total PCDF content is provided in Table 4-6, the congener concentrations still not being available. The concentrations of the toxic 2,3,7,8-TCDF and the 2,3,4,7,8-PeCDF are given in Table 4-7. Masuda et al. (1982) also found 0.01-0.02 ppm of the 2,3,4,6,7-PeCDF, ~0.01 ppm HxCDF and 0.04-0.05 ppm of an unidentified TCDF. The PCDF isomers in the rice oils exposing people in both Yusho and Yu-Cheng episodes were identical but the concentration in the Taiwan rice oil was at most 5% of the Japanese concentration. Rappe et al. (1983) detected PCDF at levels of 1-18 ppm in Kanechlor mixtures 300-600. More recently, using a complete set of PCDF standards and an isomer-specific analytical method, Rappe et al. (1984) reported on levels of 2,3,7,8-substituted PCDF congeners found in a large number of commercial PCB products (Table 4-8).

Concentrations of PCDFs in PCBs are indicative in some cases of the original concentrations of PCDFs. However, Buser et al. (1978a) demonstrated that after 2 years of using Mitsubishi-Monsanto PCB as a heat exchanger, a 4-fold increase in PCDF concentration occurred, the toxic 2,3,7,8-TCDF being the major component.

4.2.3. PCDFs in Phenoxy Herbicides. Phenoxy herbicides are widely used for the control of wood and herbaceous weeds by spraying from the air or from the ground. They reach aquatic systems directly when applied for macrophyte control in lakes, ponds and irrigation ditches or indirectly during application near water, or through runoff, reaching concentrations as high as 1-3 mg/ℓ of water (CNRC, 1978a). A 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T (Agent Orange) was used for military purposes (defoliation or crop destruction) in South Vietnam (NAS, 1974). The phenoxy

TABLE 4-8

PCDFs in Commercial PCBs (ng/g)*

PCB-typ	Tri-	Tetra-		Penta-			Hexa-					Hepta-	
	Total	2378	Total	12348 12378	23478	Total	123479 123478	123678	123789	234678	Total	Total	Rec 2378-TCDD-%
Pyralene	700	53	630	10	T	35	ND	ND	ND	ND	ND	ND	79
A1254	63	19	1,400	690	490	4000	2500	2100	190	130	10,000	960	78
A1260	10	13	110	48	56	260	500	120	190	27	1,500	1300	88
A30	500	35	573	14	28	160	50	59	ND	ND	220	T	79
A40	1300	180	2,600	96	8	1700	79	68	ND	T	310	ND	79
A50	7400	3300	20,000	760	1100	8000	700	360	18	98	3,100	75	95
A60	770	840	6,900	1100	990	8100	1600	330	170	330	6,800	2000	95
T64	47	23	360	97	122	840	520	390	58	41	2,600	220	72
Clophen C	710	54	1,200	34	30	270	Nd	T	ND	ND	T	ND	79
Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	90
Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	87

*Source: Rappe et al., 1984

ND = Not detected

herbicides, 2,4-D, 2,4,5-T, MCPA, 2-(2,4,5-trichlorophenoxy)propionic acid (Silvex) and 2-(2,4-dichlorophenoxy)propionic acid (Diclorprop) are synthesized by condensing the appropriate haloalkanoic acid with the appropriate chlorophenol, usually under alkaline conditions during reflux. Any PCDD and PCDF impurities remain in the product unless the latter is purified. In addition, since PCDDs and PCDFs are formed from chlorophenols under hot alkaline conditions, these contaminants may also be formed in the presence of excess chlorophenol.

Most attention has been accorded to the content of 2,3,7,8-TCDD in these formulations, but PCDFs have also been detected in these products (Rappe et al., 1978c; Huckins et al., 1978; Ahling et al., 1977). The analytical techniques, however, were not refined enough to permit identification of specific isomers. This has now been accomplished (Rappe et al., 1978c; Cochrane et al., 1983). The major PCDDs present in 2,4-D are the comparatively nontoxic 2,7-DCDD-, 1,3,7-TrCDD-, 1,3,4,8-TCDD and 1,3,6,8-TCDD.

Various 2,4,5-T formulations of European origin in the 1960s contained 2,3,7,8-TCDD at levels ranging from 0.1-1 μg/g as the predominant PCDD (Table 4-9). Two samples showing the presence of TCDFs did not contain the 2,3,7,8-TCDD. In the Agent Orange samples, the major PCDD was the 2,3,7,8-TCDD (0.12-5.1 μg/g levels). Only one sample contained PCDFs; they were one tri-, four tetra- and one penta-CDFs at a total level of 0.7 μg/g. The 2,3,7,8-TCDF was not present. All samples, however, showed the presence of chlorinated diphenyl ethers, which are known precursors of PCDFs in pyrolytic reactions. The chlorinated diphenyl ether content may range as high as 1% (Rappe et al., 1978c). The PCDFs may be formed from a metal ion-promoted condensation of two 2,4,6-trichlorophenolate ions, by a mechanism similar to the palladium (II) acetate synthetic method discussed

TABLE 4-9

PCDFs and PCDDs in Phenoxy Herbicides

Phenoxy Herbicide	PCDF/PCDD Amounts (mg/kg)																Reference
	Di-		Tri-		Tetra-		Penta-		Hexa-		Hepta-		Octa-		Total		
	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	
2,4,5-T Esters																	
Butyl	-	-	-	0.01	-	0.95	-	-	-	-	a	a	a	a	-	0.96	1
Isooctyl	-	-	-	-	0.15	0.18	-	-	-	-	a	a	a	a	0.15	0.18	1
Butoxyethyl	-	-	-	-	-	-	-	-	-	-	a	a	a	a	0.11	0.22	1
Butoxyethyl	a	a	a	a	0.13	0.018	0.008	0.007	<0.005	<0.0	<0.005	<0.005	<0.005	<0.005	<0.153	<0.40	2
Agent Orange	-	0.15	-	0.02		0.12	-	-	-	-	a	a	a	a	-	0.29	1
	-	0.15	0.10	0.50	0.40	1.1	0.20	0.05	-	-	a	a	a	a	0.70	1.80	1
	-	-		0.02	-	5.1	-	-	-	-	a	a	a	a	-	5.12	1

a = Not analyzed for; - = Below detection limit

1 = Rappe et al., 1978c

2 = Ahling et al., 1977

previously. In fact, it is known that the chlorinated diphenyl ethers and PCDFs are formed at the beginning of the reaction before most of the 1,2,4,5-tetrachlorobenzene reacts, which is used as starting material for the preparation of 2,4,5-trichlorophenol in the European manufacturing process; PCDDs tend to be formed near the end of the process when 2,4,5-trichlorophenolate formation is favored (Rappe, 1978; Adamoli et al., 1978). The synthesis of PCDFs from chlorobenzenes is also discussed in Section 2.5.2.2.

The concentration of the 2,3,7,8-TCDD in technical 2,4,5-T used in Agent Orange manufactured between 1958 and 1969 ranged from 1-32 ppm (Young et al., 1978). Levels in Agent Purple (n-butyl 2,4-D: n-butyl 2,4,5-T: isobutyl 2,4,5-T:: 5:3:2) formulated in the mid-1950s appear to be even higher than those in Agent Orange. Levels of 2,3,7,8-TCDD in the technical 2,4,5-T used for its formulation have been estimated to be as high as 90 ppm (Young et al., 1978). Other herbicides containing 2,4,5-T used in Vietnam, namely, Agent Pink (n-butyl 2,4,5-T/isobutyl 2,4,5-T:: 3:2), Agent Green (n-butyl ester of 2,4,5-T), Dinoxol (1:1 butoxyethanol esters of 2,4,5-T and 2,4-D), and Trinoxol (40% butoxyethanol ester of 2,4,5-T), have never been intensively studied for their PCDD or PCDF contents. However, only minor amounts of PCDF and of other PCDD in relation to the major contaminant TCDD, could be found in these herbicides. In >450 samples analyzed, TCDD had the mean value of 1.98 μg/g (Young et al., 1975, 1983). As a result of governmental regulation, efforts have been made to minimize the formation of 2,3,7,8-TCDD and now the products are estimated to contain <0.1 μg/g.

4.2.4. PCDFs in Chlorodiphenyl Ether Herbicides (PCDPE). Yamagishi et al. (1981) reported on the occurrence of PCDD and PCDF in the commercial diphenyl ether herbicides CNP, NIP and X-52. The levels of the various isomers are reported in Table 4-10.

TABLE 4-10

Levels of PCDDs and PCDFs in Commercial Diphenyl Ether Herbicides* (μg/g)

	CNP	NIP	X-52
TrCDDs	ND	0.15	0.03
TCDDs	14.00	0.38	0.03
PeCDDs	37.00	0.05	0.01
HxCDDs	0.80	ND	ND
MCDFs	ND	0.34	0.48
DCDFs	0.35	0.12	0.21
TrCDFs	0.41	0.47	0.45
TCDFs	0.40	0.29	0.32
PeCDFs	1.00	ND	0.08
HxCDFs	0.20	ND	ND

*Source: Yamagishi et al., 1981

ND = Not detected

4.2.5. PCDFs in Hexachlorobenzene. Hexachlorobenzene is a contaminant in the production of many chemicals, such as tetrachloroethylene, trichloroethylene, carbon tetrachloride, chlorine, vinyl chloride, dimethyltetrachloroterephthalate, Atrazine®, Simazine®, pentachloronitrobenzene and Mirex®. Hexachlorobenzene is also used as an economic poison for the control of wheat bunt and fungi. As an environmental contaminant it has been found in river water samples, drinking water, sewage treatment plants, effluent waters from various chemical plants, urban rain water runoff (up to 339 ng/ℓ) and surface waters (2.5 mg/ℓ) (WHO, 1979). Villaneuva et al. (1974), analyzing three commercial hexachlorobenzene preparations, identified OCDD, HpCDF and OCDF. This last compound was present in concentrations ranging from 0.35-58.3 ppm. These data suggest that PCDFs may also be found in association with hexachlorobenzene.

4.2.6. PCDFs in Hexachlorophene. The bactericide, hexachlorophene, is prepared commercially from 2,4,5-trichlorophenol (Nilsson et al., 1978). It is purified, however, during this process and levels of 2,3,7,8-TCDD are <30 μg/kg. Nevertheless, hexachlorophene can contain 100 ppm of the 1,2,4,6,8,9-hexachloroxanthene (Gothe and Wachtmeister, 1972).

4.2.7. PCDFs in PBBs. The PBBs gained their greatest notoriety as a result of a severe contamination of meat and dairy products in Michigan in October, 1973 (Cordle et al., 1978). The contaminant, Firemaster BP-6 manufactured by the Michigan Chemical Corporation, contained 2% tetrabromobiphenyls, 10.6% pentabromobiphenyls, 62.8% hexabromobiphenyls, 13.8% heptabromobiphenyls and 11.4% other bromobiphenyls. As a result of error, Firemaster BP-6 was added to cattle feed instead of magnesium oxide. The levels of contamination included the following: lot 405, 2.4 ppm; lot 410, 1790 ppm; and lot 407, 4300 ppm. Milk from affected herds contained from 2.8-271 ppm on a fat basis. Contaminated butter (1-2 ppm), cheese (1.3-15 ppm) and canned milk (1.2-1.6 ppm) were later seized and destroyed.

PBDFs were found in pyrolyzed PBBs by O'Keefe (1978b) and Buser et al. (1978a). PBDFs were not found in one study on unheated Firemaster FF-1 (Hass et al., 1978). Hass et al. (1978) concluded that the amount of BDBFs must be <0.5 ppm, and that penta- and hexabromonaphthalenes were present to the extent of 150 and 70 ppm, respectively. A metabolite of Firemaster BP-6 in dogs, 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl, decomposed at 230-260°C on an OV-101 column in a gas chromatograph to form two PeBDFs (Gardner et al., 1979). Thus, PBDFs can be formed from pyrolysis on GC columns as well as being originally present in PBB formulations. Such artifacts need to be investigated before PBDF contamination of PBB formulations is considered confirmed.

4.2.8. PCDFs in Metal Salts. Salts of metals often used in industrial processes may contain PCDFs and PCDDs (Heindl and Hutzinger, 1986). Table 4-11 shows that the chlorides of iron, aluminum and copper contain between 0.02 and 60 ppb PCDFs and 0.02-0.63 ppt PCDDS, both mostly as OCDF and HpCDF.

4.3. PHOTOCHEMICAL PRODUCTION

Another potential source of PCDFs derives from the photodegradation reactions of a number of chemicals present in the ecosystem as pollutants. Perhaps the most important potential source of environmental contamination is the photochemical production of PCDF from PCBs. Hutzinger (1972) observed 0.2% 2-PCDF production after 7 days of ultraviolet irradiation (310 nm) of aqueous solutions of 2,5,2'5'-tetrachloro- and 2,5-dichloro-biphenyls (5 mg/ℓ). These products were confirmed by Crosby and Moilanen (1973) (see Section 2.4.2. for more details of laboratory photochemistry).

TABLE 4-11

PCDFs and PCDDs in Several Metal Salts*
(ppb)

Salt	OCDF	HpCDF	OCDD	HpCDD
$FeCl_3$	42	12	<0.02	<0.02
$AlCl_3$ (A)	<0.02	<0.02	<0.02	<0.02
$AlCl_3$ (B)	34	0.1	<0.02	<0.02
$CuCl_2$	0.5	0.1	0.6	0.03
CuCl	0.2	0.08	0.03	<0.02
$TiCl_4$	<0.02	<0.02	<0.02	<0.02
$SiCl_4$	<0.02	<0.02	<0.02	<0.02

*Source: Heindl and Hutzinger, 1986

However, Hutzinger (1972) found no PCDFs after some aqueous PCB samples (167 mg/ℓ) had been exposed to sunlight for >2 months. This discrepancy may have arisen because of the different irradiation times and different wavelengths used. The yield of the products was wavelength dependent. Irradiation at 254 nm (mercury arc) decomposed PCDF photoproducts but only slowly at 310 nm or at the wavelengths encountered at sea level for sunlight, or for a solar simulator. Triplet sensitizers (for example, 4,4'-dichlorobenzophenone) induced faster decomposition as observed in methanolic solution alone for the photodecomposition of 2,8-DCDF (Crosby and Moilanen, 1973). Thus, the presence of other compounds may be important.

After ultraviolet irradiation of some chlorophenols, Crosby et al. (1973) detected OCDD but PCDFs were not sought. Chlorinated o-phenoxyphenols (predioxins), which are common impurities in chlorophenols (1-5%), also produce PCDDs after ultraviolet irradiation (Nilsson et al., 1974) but again PCDFs were not sought specifically. Another source of PCDFs through photodecomposition is from PCDPEs, which are often present at levels of 100 ppm in commercial chlorophenols. Irradiation of 100 ppm solutios in methanol, ethanol or n-hexane with light of 248-579 nm caused cyclization in diphenyl ethers containing at least one ortho-chlorine. Reductive dehalogenation was enhanced in hexane. Although no yields were explicitly given, the degradation was characterized by an induction period and then decomposition, obeying a first-order kinetic law (Norstrom et al., 1976a, 1977; Choudhry et al., 1977a,b). Photodegradation of polychlorobenzenes (PCBZ) can also be a source of PCDF production. Laboratory irradiation at $\lambda \geq 285$ nm of a solution of PCBZ in the presence of phenol yielded PCDF ($\leq 1\%$) containing two Cl atoms less than the starting PCBZ. This reaction probably proceeds through the intermediacy of PCDPE (Choudhry et al., 1983). The relevance of these laboratory photodecompositions to environmental sources of PCDFs is unknown.

Another photochemical process of potential environmental importance is dechlorination of the higher PCDFs. This topic will be considered in Chapter 5.

4.4. SOURCES OF PCDF FROM BURNING AND OTHER HIGH TEMPERATURE PROCESSES

In 1978 Dow Chemical scientists proposed the "Trace chemistries of fire" hypothesis. This stated that many contaminants arise in trace amounts during chemical reactions during combustion. PCDF and PCDD are included among these compounds. To assess the relative importance of these sources, Sheffield (1985a,b) estimated the environmental release of PCDF and PCDD from combustion sources in Canada (Table 4-12) using the small data base at his disposal.

4.4.1. Thermal Degradation of Technical Products.

PCDFs can arise from pyrolysis of specific PCBs, chlorinated benzenes and chlorinated diphenyl ethers, and PBDFs from the analogous brominated precursors. The occurrence of these compounds must be taken into consideration since they are released into the environment with their co-contaminants and may produce PCDFs upon incineration.

The levels of PCDFs in PCBs increase with the length of time in service at high temperatures as heat exchange media (Morita et al., 1977b; Buser et al., 1978d), as originally suggested by Kuratsune et al. (1976). A contrary finding has been reported by Cull and Dobbs (1984). Aroclor 1254 on pyrolysis also contained the major toxic PCDFs, but the relative amounts of the products differed somewhat from those obtained from the Mitsubishi-Monsanto T-1248 (Buser and Rappe, 1979). The major PCDFs identified in the used

TABLE 4-12

Estimated PCDD and PCDF Releases to the Canadian Environment from Combustion Sources[a]

Sources	Release (g/year)	
	PCDD	PCDF
Municipal incinerator		
Fly ash	2900-7100	4900-15,600
Air emission	250-13,700	550-21,700
Sewage sludge incineration		
Air emission	1400-3300	1500-6500
Coal-fired utility boilers		
Fly ash	ND-300	30-1300
Air emission	300	700
Fuelwood combustion		
Air emission	1800	not estimated
Residential oil combustion		
Air emission	<1	not estimated
Residential gas combustion		
Air emission	900	not estimated
Wigwam burners/Wood waste boilers		
Air emission	0-30,200[b]	
Railway ties		
Air emission	6000[b]	
Forest fires		
Air emission	58,700	not estimated
Slash burning		
Air emission	3300	not estimated
Cigarette smoke		
Air emission	2-4	not estimated
Motor vehicles		
Air emission	200	not estimated

[a] Source: Sheffield, 1985a,b

[b] PCDD + PCDF release

ND = Not detectable

Mitsubishi-Monsanto T-1248 were the 2,3,7,8-TCDF (1.25 ppm), the 2,3,4,7,8-PeCDF (the pyrolysis product of 2,4,5,2',4',5'-hexachlorobiphenyl), 1,2,3,7,8-PeCDF, 2,3,4,6,8-PeCDF, 1,2,3,4,8-PeCDF, 1,3,4,7,8-PeCDF (also from pyrolysis of 2,4,5,2',4',5'-hexachlorobiphenyl) and the 2,3,4,6,7,8-HxCDF. The 2,3,4,8- and 2,3,7,8-TCDFs were later shown to co-elute (Mazer et al., 1983a,b; Rappe et al., 1984). Whereas the used Mitsubishi-Monsanto T-1248 was exposed for years to elevated temperatures in the liquid phase, the laboratory pyrolyses of Aroclor 1254 and 1260 were performed in the gas phase for a few seconds up to a maximum temperature of 700°C (Buser and Rappe, 1979). With Aroclor 1254 (tri- to hepta-PCBs), mostly MCDFs to PeCDFs were formed at a level of ~2%. With Aroclor 1260 (penta- to octa-PCBs), mostly TrCDFs to HpCDFs were produced at a similar level. The toxic 2,3,7,8-TCDF was the most abundant TCDF, most likely derived from 2,4,5,2',4',5'-hexachlorobiphenyl or 2,4,5,3',4'-pentachlorobiphenyl. The acutely toxic 1,2,3,7,8- and 2,3,4,7,8-PeCDFs were the major PeCDFs. The former probably is produced from pyrolysis of 2,3,4,2',4',5'-hexachlorobiphenyl, and the latter from 2,4,5,2',4',5'-hexa- or 2,3,4,5,2',4',5'-heptachlorobiphenyl. Pyrolyzed Aroclor 1254 also contained significant amounts of 1,3,4,7,9-PeCDF. These results were confirmed by Paasivirta et al. (1985) who also found small amounts of chlorinated phenols, naphthalenes, and MCDFs and DCDFs (Table 4-13) produced between 500 and 700°C.

The polybrominated biphenyl, Firemaster FF-1, when pyrolyzed for 20 minutes at 380-400°C in open glass tubes, produced 40 ppm TBDFs and 4 ppm PeBDFs based on PBB content. Only trace amounts (~1 ppm) were found if the pyrolysis was redone in a nitrogen atmosphere (O'Keefe, 1978b). It was postulated that since 2,4,5,2',4',5'-hexabromobiphenyl was the major hexabromobiphenyl present, nearly all of the tetrabromo-isomer was the 2,3,7,8-isomer. The larger yield is expected from the weaker ring carbon-bromine

TABLE 4-13

Pyrolysis Products of Aroclor 1254 in a Quartz Tube for Pyrolysis Time of 3 Seconds*
The Carbon Filter Adsorbed any Volatilized PCB
[relative amounts (%)]

Pyrolysis Temperature (%)	PCDF						PCDD			
	Cl_1	Cl_2	Cl_3	Cl_4	Cl_5	Cl_6	Cl_2	Cl_3	Cl_4	Cl_5
500	0.1	14	39	40	7.4	ND	17	10	73	ND
600	0.3	13	39	36	11	0.8	0.7	18	57	24
700	0.01	5.2	29	49	15	1.5	4.6	14	66	17
Filter	52	44	3.9	ND	ND	ND	NA	NA	NA	NA

*Source: Paasiverta et al., 1985

ND = Not detected

NA = Not analysed

bond relative to that of carbon-chlorine. The production of PBDFs from various PBBs in sealed ampules was also studied by Buser et al. (1978a) and has been discussed in Section 4.2.5.1. The 10-minute pyrolysis of 2,4,6-tribromophenol, pentabromophenol, tetrabromobisphenol A and tetrabromophthalic anhydride, all used as flame retardants, resulted in PBDD and PBDF between 700 and 900°C, except for the anhydride that did not form PBDFs/PBDDs at any of the three temperatures. In general, the optimum temperature to form PBDDs and PBDFs was at 800°C; 2,4,6-tribromophenol pyrolysis produced 8950 ppm TBDFs, pentabromophenol formed 7042 ppm HpBDFs with no detectable amounts of the other PBDF congeners. Small amounts were noted at 900°C, although lower congeners did appear at 700°C. The tetrabromobisphenol A still produced relatively high PBDF concentrations at 900°C and PBDFs predominated over PBDDs unlike at other temperatures and for the other two phenols when they were pyrolyzed. The absence of any PBDDs or PBDFs from pyrolysis of the anhydride indicates that such compounds will be safer flame retardants.

Chlorophenolates burned for 15 minutes on birch leaves or wood wool or heated at 280°C for 30 minutes (Table 4-14) evolved PCDDs, but not many PCDFs (Rappe et al., 1978b). Originally, both types of formulations studied (Servarex Teknisk and Kymmene KY-5, both containing 5% 2,4,6-tri-, 50% 2,3,4,6-tetra- and 10% pentachlorophenols as the sodium salts) contained 10 ppm each of tetra-, penta- and octa-CDFs and 70 ppm each of the hexa- and hepta-CDFs, with 40-50 isomers present (see Table 4-4). The burnt samples produced only 10 isomers and levels were lower than the original levels (see Table 4-14). Nevertheless, the major TCDF (unknown) increased 100-fold during burning (from 0.04-5 ppm) and two others that were not in the original formulation were detected. The 2,3,7,8-isomer was a very minor

TABLE 4-14

Levels of Trapped PCDFs and PCDDs on Charcoal from Burning Chlorophenolate-Impregnated Leaves and Wood Wool[a]

Commercial Chlorophenol	Substrate	μg PCDF or PCDD/g Chlorophenate									
		Tetra-		Penta-		Hexa-		Hepta-		Octa-	
		CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD
2,3,4,6-Tetra- (Servarex)	Birch leaves	$<10^b$	35	$<10^b$	90	$<70^b$	80	$<70^b$	8	$<10^b$	0.3
	Wood wool	$<10^b$	96	$<10^b$	120	$<70^b$	110	$<70^b$	65	$<10^b$	1.2
(Pure)		$<10^b$	30	$<10^b$	84	$<70^b$	82	$<70^b$	8	<10b	0.4
2,4,6-Trichloro-	Birch leaves	--	2100	--	5	--	1	--	3	--	6
Pentachloro-	Birch leaves	--	5	--	14	--	56	--	172	--	710

[a] Source: Rappe et al., 1978b

[b] Signifies the amount in the original formulation

component in the starting material (0.1% of the total PCDFs). When the pure phenols were micropyrolyzed, no PCDFs could be detected. Thus, the newly formed PCDFs had to arise from impurities in the technical products (for example, PCBs or probably PCDPEs) (Lindahl et al., 1980). K_{12} formulations were particularly contaminated by PCDF/PCDD contaminants, but K_1 and Ky-5 types contained lower levels (Paasiverta et al., 1982). Polychlorinated phenoxybipheny diols and polychlorinated phenoxyphenols were also detected. Ky-5 burnt in an open fire caused enhanced levels of all PCDDs/PCDFs in the residual ash and in smoke vapor with disappearance of PCDEs from the Ky-5 formulation. The PCDDs probably arose from the polychlorinated phenoxyphenol and chlorophenol contaminants.

Samples of ash from two airtight wood burning stoves, one open fireplace, and after outside open-air burning and from associated chimney ash contained traces of PCDDs and PCDFs (total PCDD or PCDF never >15 ng/g). PeCDFs to HpCDFs dominated. Unfortunately, the recovery of 1,2,3,4-TCDD varied between 15 and 80% and of OCDD between 41 and 92%. The past history of the wood was not documented fully (Clement et al., 1985b).

The production of PCDDs and PCDFs from the 2-butoxyethyl ester of 2,4,5-T was studied by pyrolyzing the ester for 0.6-1 second when the ester (1 kg) was mixed with leaves or sawmill wood chips (Ahling et al., 1977). The PCDF levels usually exceeded PCDD levels (Table 4-15) but not between 100 and 625°C, even though PCDFs were more concentrated in the original formulation (which also contained 47 mg/g 2,4,5-T, 230 μg/g of 2,4,5-trichlorophenol and 12 μg/g PCP in addition to the PCDFs and PCDDs cited in Table 4-15). It was thought that the chlorophenols could not be the source of the PCDDs or PCDFs but that a complex mechanism was involved.

TABLE 4-15

Production of PCDDs and PCDFs after 0.6 to 1.00 Second Pyrolysis in Air of 2-Butoxyethyl-2,4,5-Trichlorophenoxyacetate*

Temperature (°C)	PCDF/PCDD Amounts(mg/kg)											
	Tetra-		Penta-		Hexa-		Hepta-		Octa-		Total	
	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD	CDF	CDD
25	0.13	0.018	0.008	0.007	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.36	0.040
100	1.0	2.5	0.5	<0.5	<0.4	<0.5	2.3	<0.5	<0.4	<0.5	4.6	4.5
500	0.4	2.2	1.5	<0.5	2.0	1.3	<0.7	<0.5	<0.7	<0.5	5.3	5.0
625	0.2	0.4	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	1.4	1.6
675	8.0	2.3	7.1	<0.5	2.8	<0.5	<0.7	<0.5	<0.7	<0.5	19.3	4.3
800	2.1	0.5	3.0	<0.3	3.3	<0.3	2.4	<0.3	2.3	<0.8	13.1	1.9

*Source: Adapted from Ahling et al., 1977

Recently direct evidence has been obtained of the formation of PCDF and PCDD from polyvinyl chloride (PVC) (Marklund et al., 1986a), as previously suggested by Ahling et al. (1978). In laboratory conditions pyrolysis of PVC results in the formation mainly of TCDF and HpCDF. The pattern of isomers appeared similar to those found in municipal and hazardous waste incinerators. The same isomeric pattern was also found in emissions from a smoke generator burning hexachloroethane (Marklund et al., 1986a), and from leaded gasoline containing 1,2-dichloroethane (Marklund et al., 1986b).

4.4.2. Incineration of Municipal Waste.

4.4.2.1. PRESENCE OF PCDFs IN INCINERATOR EMISSIONS -- Olie et al. (1977) detected the presence of trace amounts of PCDDs and PCDFs in fly ash and flue gas of some municipal incinerators in The Netherlands. The presence of PCDDs and PCDFs in fly ash from a municipal and an industrial incinerator was later confirmed by Buser and Bosshard (1978) and by Eiceman et al. (1979). Reports and reviews from many countries (Samuelson and Lindskog, 1983; Chiu et al., 1983; Ballschmiter et al., 1983; Janssens et al., 1982; Sheffield, 1985a,b) have since been published, confirming the previous results. The average PCDF concentration for total stack effluents is ~2.8 μg/m^3. However, the terms of total levels including those of congeners may not be correlated to potential toxicity.

Buser and Bosshardt (1978) and Buser et al. (1978b) suggested that the presence of PCDFs in the fly ash of municipal incinerators could be explained by the pyrolysis of PCBs. Fly ash samples from an industrial heating facility at Aarau and a municipal incinerator in Zurich, both in Switzerland, were analyzed. PCDDs and PCDFs were found in both locations with levels of 0.3 and 0.1 ppm for the Aarau and Zurich samples, respectively, and were 0.33-0.50 the total PCDDs present. The levels of PCBs were the same as for PCDFs. Polychlorinated naphthalenes were also present in smaller quantities. The PCDF congener profile from fly ash was very similar to that from pyrolyzed commercial PCBs, suggestive of a common source. PCDFs were detected as follows: 18 tri-, 21 tetra- (mainly the 2,3,7,8-), 17 penta- (including the acutely toxic 1,2,3,7,8- and 2,3,4,6,8-), 7 hexa- and 4 hepta- (1,2,3,4,6,7,8- most abundant). Fly ash samples contained large amounts of 2,3,4,6,8- and 1,2,3,4,8-penta- and other penta-isomers relative to pyrolyzed Aroclor.

Olie et al. (1977) also detected large amounts of chlorobenzenes and chlorophenols in the fly ash of municipal incinerators in The Netherlands, but provided no quantitative data. The Dow Chemical Company has suggested that PCDDs are ubiquitous products of the combustion of all chlorinated material, including that found in municipal incinerators (Bumb et al., 1980; Crummett and Townsend, 1984).

A summary of the large amount of work done since the initial findings is provided in Tables 4-16 and 4-17 for incinerator fly ash and stack effluent, respectively. Lustenhouwer et al. (1980) postulated that polyvinyl chloride pyrolysis produced chlorobenzenes in small amounts, and these could further pyrolyze to form PCDDs and PCDFs. Olie et al. (1983) found that PCDDs and PCDFs did not increase when hexachlorobenzene, wood treated with pentachlorophenol, paper treated with hypochlorite, old painted wood or lignin sulfonate/polyvinylchloride were burnt. However, lignin sulfonate/HCl burning caused a marked increase. Nevertheless, Liberti and Brocco (1982) showed that decreased levels of PCDDs/PCDFs occurred for wastes pretreated by the Dano process or if paper, plastic and vegetable matter were removed. Table 4-18 depicts the influence of type of waste on PCDD/PCDF content.

TABLE 4-16

PCDF Composition of Municipal Incinerator Fly Ash Resulting from Electrostatic Precipitation

Origin of Fly Ash[a]	PCDF (ng/g)							Reference
	Recovery (%)	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Arnhem, The Netherlands (1)	NS	NM	trace	trace	trace	trace	trace	Olle et al., 1977
Alkmaar, The Netherlands (1)		NM	trace	trace	trace	trace	ND	
Unknown, Switzerland (1)	NS	NM	1	4	30	40	10	Buser et al., 1978d
Ontario Canada (1)		NM	3272	5697	1415	967	ND	Eiceman et al., 1979
Japan (1)	NS	NM	789	3691	1069	2389	1120	
Japan (1)		NM	ND	3827	ND	2555	2341	
The Netherlands (1)		NM	ND	ND	ND	ND	ND	
Unknown (25)	100	NM	13-223	42-510	109-870	89-407	20-26	Lustenhouwer et al., 1980
One incinerator over 4 weeks	100	NM	50-147	153-239	282-361	177-291	18-31	
Milan, Italy (1)	NS	NM	NM	NM	NM	NM	166	Cavallaro et al., 1980
Milan, Italy (1)	NS	NM	NM	NM	NM	NM	6-22	
Busto, Italy (1)	NS	NM	NM	NM	NM	NM	0-18	
Desio, Italy (1)	NS	NM	NM	NM	NM	NM	70	
Unknown (1)	100	NM	169	364	555	287	32	Kooke et al., 1981
Unknown (2)	100	NM	223-315	510-560	840-870	407-495	84-89	
Bologne, Italy (1)	NM	NM	112	205	235	345	425	Liberti and Brocco, 1982
Milan, Italy (1)		NM	51	115	177	310	547	
Alkmaar, The Netherlands (17 samples over 5 months)	80[b]	NM	76-410	165-364	160-1260	108-300	0-126	Olle et al., 1982
Amsterdam, The Netherlands (14 samples over 18 monihs)		NM	12-184	29-122	29-287	71-89	2-97	
Zaanstad, The Netherlands								
(24 samples over 20 months)		NM	20-471	31-792	82-1054	47-714	4-223	
(8 samples every 20 minutes over 2.5 hours)		NM	118-291	222-568	566-1310	397-699	37-145	
West Germany (1) (rotary kiln-33 samples)	NS	NM	0.8-28	0.3-8	0.4-8	0.6-10	0.3-14	Karasek and Viau, 1983

TABLE 4-16 (cont.)

Origin of Fly Ash[a]	PCDF (ng/g)							Reference
	Recovery (%)	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
USA (1)	50-100	NM	419	107	263	287	25	Taylor et al., 1983
Florence, Italy (1) (3 laboratories)	NS	NM	3.2	22	35	9-51	10-230	Brocco et al., 1984
U.S. cyclone ash (1) U.S. fly ash (1)	75 75	NM NM	50 50	75 100	170 400	80 300	5 20	Czuczwa and Hites, 1984
Ontario, Canada (1)	90	NM	294	508	420	428	101	Tong et al., 1984
U.S. (1) (2 samples)	NS	520-580	350-460	1860	75-91	9-11	0.57-2	Haile et al., 1985
			(Cl_1 = 40-42; Cl_2 = 69-110)					
Vienna, Austria (1)	NS	NM	39	60	154	156	168	Scheidl et al., 1985
Ehime, Japan 8 cities	50	0.7-32	0.4-31	ND-180	trace to 56	0.1-11	ND-23	Wakimoto and Tatsukawa, 1985
cinders	50	0.4-14	0.1-84	trace to 12	ND-25	ND-0.8	ND-0.9	
Ontario, Canada	NS	NM	965	1865	1990	850	ND	Shushan et al., 1985
USA	NS	NM	26-150	52-390	120-880	72-380	8-31	Clement et al., 1985a

[a] Number in parenthesis

[b] 1,2,3,4-TCDD

NS = Not stated

NM = Not measured

ND = Not detected

TABLE 4-17

PCDF Composition of Stack Effluent of Municipal Incinerators

Origin of Study Emission/Number	PCDF (ng/m³)							Reference
	Recovery (%)	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Unknown, Switzerland	NS	NM	trace	trace	trace	NM	NM	Buser et al., 1978c
Unknown	100[a]	NM	460[b]	960[b]	1600[b]	1130[b]	140[b]	Lustenhouwer et al., 1980
Milan, Italy (5 samples)	NS[a]	NM	NM	NM	NM	NM	20-47[b]	Cavallaro et al., 1980
	c/	NM	NM	NM	NM	NM	270-1080	
Milan, Italy (4 samples)	NS[a]	NM	NM	NM	NM	NM	0.9[b]	
	c/	NM	NM	NM	NM	NM	90	
Busto, Italy	NS[a]	NM	NM	NM	NM	NM	4-70[b]	
	c/	NM	NM	NM	NM	NM	5-10	
Desio, Italy	NS[a]	NM	NMN	NM	NM	NM	10[b]	
	c/	NM	NM	NM	NM	NM	51	
Valmadrera, Italy (16 samples over 9 months)	85[d,e]	NM	17-2846 309[f]	17-2261 250[f]	22-2928 314[f]	17-1414 215[f]	17-749 124[f]	Gizzi et al., 1982
Florence, Italy	NS[d]	NM	175[b]	240[b]	185[b]	400[b]	570[b]	Liberti and Brocco, 1982
	c/	NM	ND	ND	125	105	31	
Zaanstad, The Netherlands (14 samples over 6 months)	80[d]	NM	46-556	109-702	174-1437	155-504	0-201	Olie et al., 1982
Unknown, U.S.A.	81[d] d/	300	90	NM	62	7.5	0.8	Redford et al., 1983
Ontario, Canada (1)	NS[a]	NM	46	153	1712	47	ND	Chiu et al., 1983
	c/	NM	8	15	ND	ND	ND	
(2)	NS[a]	NM	279	265	392	104	ND	
	c/	NM	5	5	ND	ND	ND	
Eksjo, Sweden (2 samples)	NS[a]	NM	25-37	4.3-10	4.3-25	8.3-25	8.3-9	Rappe et al., 1983a
	c/	NM	68-525	5.7-87	10-80	7.3-15	1.3-5.7	
Florence, Italy (3 samples)	NS[a]	NM	NM	NM	NM	NM	168-498	Brocco et al., 1984
	c/	NM	NM	NM	NM	NM	411-546	

TABLE 4-17 (cont.)

Origin of Study Emission/Number	PCDF (ng/m³)							Reference
	Recovery (%)	Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Germany (1)	NS[a]	NM	2^h	NM	NM	NM	7^h	Ballschmiter et al., 1984, 1985
	c/	NM	98^h	NM	NM	NM	93^h	
	a/	NM	3^h	NM	NM	NM	63^h	
	c/	NM	98^h	NM	NM	NM	37^h	
	a/	NM	18^h	NM	NM	NM	81^h	
	c/	NM	82^h	NM	NM	NM	19^h	
	a/	NM	59^h	NM	NM	NM	67^h	
	c/	NM	41^h	NM	NM	NM	33^h	
	a/	NM	9^h	NM	NM	NM	100^h	
	c/	NM	91^h	NM	NM	NM	$<1^h$	
	a/	NM	2^h	NM	NM	NM	3^h	
	c/	NM	98^h	NM	NM	NM	97^h	
U.S. (1) (5 days)	d/	1100-3300 (Cl_1 = 300-420; Cl_2 = 400-700)	480-2000	1300-15,000	170-1800	83-380	8-24	Halle et al., 1985
Vienna, Austria (1)	d/	NM	100	120	150	110	30	Scheidl et al., 1985
Quebec, Canada (3)	d/	NM	ND-142	ND-124	ND-1392	ND-123	ND-3	Sheffield, 1985a,b
Ontario, Canada (2)	d/	NM	53-4850	64-3960	152-2120	20-880	10-170	

[a] Particulate

[b] ng/g

[c] Signifies "condensate"

[d] Combined a and c

[e] 2,3,7,8-TCDD

[f] Arithmetic means

[g] 1,2,3,4-TCDD

[h] In % of isomeric class

NS = Not stated; NM = not measured; ND = not detected

TABLE 4-18

Influence of Type of Waste on PCDF Formation

Waste	Substrate	PCDF (ng/g)						Reference
		Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Untreated	bottom ash	NM	NM	NM	NM	25	50	Liberti and Brocco, 1982
Urban Waste	fly ash	NM	NM	NM	NM	70	50	
Agricultural Waste	fly ash	NM	NM	NM	NM	ND	ND	
	sludge	NM	NM	NM	NM	ND	ND	
Recycled waste[a]	sludge	NM	NM	NM	NM	ND	ND	
Unfermented waste surviving the Dano process	particulate stack emission	NM	NM	NM	NM	ND	ND	
	sludge	NM	NM	NM	NM	ND	ND	
Coal-fired power plant, U.S.A.	stack emission	ND	ND	ND	ND	ND	ND	Haile et al., 1983
	fly ash							Redford et al., 1983
Coal-fired power plant, Canada	fly ash	NM	8	32	18	15	ND	Chiu et al., 1983
Low temperature incinerator	bottom ash	NM	0.3	1.1	0.4	0.1	0.1	
Pentachlorophenol impregnated waste	particulate	NM	ND	ND	ND	ND	25	
	condensate	NM	ND	ND	ND	ND	ND	
Plasma Pyrolysis of Aroclor 1254	condensate	NM	0.9	4.8	9.1	18	18	

TABLE 4-18 (cont.)

Waste	Substrate	PCDF (ng/g)						Reference
		Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Pentachlorophenol	bottom ash	NM	ND	ND	ND	ND	ND	Rappe et al., 1983a
Contaminated waste	baghouse ash	NM	900	1500	150	60	6	
PCB incinerator in	particulate	NM	ND	ND	ND	ND	ND	
rotary cement kiln	condensate	NM	ND	ND	ND	ND	ND	
Low sulfur coal	fly ash	NM	ND	ND	2	4.2	0.1	Czuczwa and Hites, 1984
		NM	ND	ND	0.1	0.2	ND	
Hazardous waste 1	stack emission	NM	22[b]	NM	NM	NM	NM	Oberg and Bergstrom, 1985
2	stack emission	NM	62[b]	NM	NM	NM	NM	
3	stack emission	NM	<0.4[b]	NM	NM	NM	NM	
Municipal waste 1	stack emission	NM	4.5[b]	NM	NM	NM	NM	
2	stack emission	NM	4[b]	NM	NM	NM	NM	
3	stack emission	NM	21[b]	NM	NM	NM	NM	
Peat 1	stack emission	NM	<0.0006[b]	NM	NM	NM	NM	
2	stack emission	NM	0.15[b]	NM	NM	NM	NM	
Coal (Canada)	fly ash	NM	ND-0.9	ND-2.8	ND-0.25	ND-0.19	ND-0.13	Sheffield, 1985a,b

[a] With paper, plastics and vegetable matter removed

[b] ng/m^3

NM = Not measured

ND = Not detected

Thus, no PCDFs/PCDDs were found in stack emissions, fly ash or coal at four coal-fired power plants (Haile et al., 1983; Redford et al., 1983). Some trace PCDDs/PCDFs were found in the stack emissions when municipal waste was burnt. The results are probably caused by optimum combustion conditions, 1200°C for the coal-fired plant and 650°C with a 20-minute residence time for the municipal waste. Levels up to a few pg/m^3 were reported from a large peat incinerator in Sweden (Marklund et al., 1986a).

There is still controversy over whether the vapor or particulate phase contains the most PCDDs/PCDFs in the stack emissions of municipal incinerators (see Table 4-17). The vapor generally appears the preferred localization phase (Cavallaro et al., 1980; Ballschmiter et al., 1984, 1985; Benfenati et al., 1986). A $^{13}C_{12}$-labeled surrogate added to the filter before sampling was found preferentially in the condensate and adsorbent (Rappe et al., 1986). Combustion in the municipal incinerators sampled occurred in the temperature range 500-1000°C with an optimum formation temperature of ~500°C (Gizzi et al., 1982).

Higher temperatures (>1400°C) may solve the PCDF residue problem as indicated by a study of the PCB, PCDF and PCDD content of the residual ash of a cement kiln. Residual PCB of ~10 mg/kg was detected for a chamber pyrolysis time of 5 seconds at 700-1000°C (Ahling and Lindskog, 1978), but this was drastically lowered at 1400-1450°C (Ahling, 1979). No PCBs were found in the flue gases, and no PCDDs or PCDFs found in the kiln residues, although peaks corresponding to the expected retention time for OCDF were noted. Municipal incinerators operating from 320-900°C favored PCDF formation over PCDD (Benfenati et al., 1983; Taylor et al., 1983). This is so for all isomer classes except for hepta- and octa- (Taylor et al., 1983).

The highest emissions also appeared to be related to the presence of HCl, but not with particulate matter (Benfenati et al., 1983). The degree of temperature control appears to be the dominating factor ($r \approx 0.839$) and not absolute efficiency of combustion/precipitation since the lowest temperature reached is the most highly correlated parameter for PCDF/PCDD formation for a given waste (Benfenati et al., 1983). Olie et al. (1982) recognized that the presence of 2,3,7,8-TCDD could not alone explain the toxicity of fly ash. They have calculated an index called the "2,3,7,8-TCDD toxic equivalents" to describe the toxicity based on the presence of toxic 2,3,7,8-substituted isomers (Table 4-19). Marklund et al. (1986a) reported the toxic TCDD equivalents (Eadon, 1982) based on the concentrations of the toxic 2,3,7,8-substituted PCDFs and PCDDs found in the stack emissions of a Swedish municipal incinerator (Table 4-20).

4.4.2.2. ORIGIN OF PCDF IN INCINERATOR EMISSIONS -- The origin of PCDF and PCDD in incinerator emissions has not been clarified. The hypothesis that the contaminants are already present in waste and are not completely transformed during combustion is generally not accepted. However, Ozvacic et al. (1984) found in raw waste an average concentration of 2-3 ng/g PCDF and 19.8 ng/g PCDD to 4 through 8 Cl-substituted. Although concentrations of the refuse are low, Tosine et al. (1985) suggest that levels of PCDD and PCDF in feedstock should not be ignored in studies designed to determine mechanisms of formation of these substances.

A second hypothesis suggests PCDF and PCDD formation from precursors such as PCB, chlorophenols and chlorobenzenes present in the raw refuse. Laboratory studies have shown that pyrolysis of a variety of chlorinated chemicals can lead to the occurrence of PCDF and PCDD (see Section 4.4.1.).

TABLE 4-19

Annual Toxic Equivalent of CDBFs Emitted by Netherlands Incinerators in Terms of 2,3,7,8-TCDD Toxic Equivalents*

Substrate	Parameter (kg)	PCDF Isomers Cl_4	PCDF Isomers Cl_5	PCDF Isomers Cl_6	PCDF Isomers Cl_7	2,3,7,8-TCDD
Fly ash	amount/year	10.4	18.7	27.5	18.8	
	toxic equivalent	0.2	0.9	2.1	0.9	0.2
Stack effluent	amount/year	2.3	3.8	7.4	4.1	
	toxic equivalent	0.048	0.19	0.56	0.21	0.026

*Source: Olie et al., 1982

TABLE 4-20

Levels of PCDD and PCDF from MSW Incineration, Umea*
(ng/Nm³ dg 10% CO_2)

Experiment	Normal	Normal Chips	Normal Oil	Low Temperature	Low Temperature Oil	Start	Start Oil	Normal
Season	Fall	Fall	Fall	Fall	Fall	Fall	Fall	Spring
Number of experiments	3	2	2	3	3	1	1	3
2,3,7,8-TCDF	2.5	2.3	2.4	2.6	2.1	9.5	2.3	0.85
Total TCDF	86	75	68	87	75	260	80	19
2,3,7,8-TCDD	0.5	0.6	0.7	0.4	0.3	1.3	0.7	<0.1
Total TCDD	43	45	52	54	47	100	49	<10
1,2,3,7,8-/ 1,2,3,4,8-PeCDF	9.0	8.3	9.8	8.3	7.1	52	9.0	2.5
2,3,4,7,8-PeCDF	6.1	7.3	7.8	7.4	6.5	40	9.0	3.9
Total PeCDF	97	100	120	110	87	520	120	43
1,2,3,7,8-PeCDD	2.5	3.6	3.6	3.2	3.6	14	3.9	2.4
Total PeCDD	53	70	76	80	70	280	90	49
1,2,3,4,7,8-/ 1,2,3,4,7,9-HxCDF	3.6	4.6	5.6	5.2	3.6	48	5.7	4.5
1,2,3,6,7,8-HxCDF	3.7	4.6	5.5	5.0	3.4	40	5.7	4.6

TABLE 4-20 (cont.)

1,2,3,7,8,9-HxCDF	0.8	0.8	1.2	1.2	1.4	52	2.3	3.6
2,3,4,6,7,8-HxCDF	2.6	4.4	4.3	5.1	4.2	36	5.4	4.3
Total HxCDF	33	46	51	50	37	380	54	43
1,2,3,4,7,8-HxCDD	1.6	2.9	3.5	5.1	3.1	31	4.4	2.3
1,2,3,7,8,9-HxCDD	3.7	5.6	6.5	9.5	6.6	56	7.9	5.8
1,2,3,7,8,9-HxCDD	1.3	2.4	3.0	3.8	2.6	20	3.5	2.0
Total HxCDD	32	53	72	82	57	400	70	55
Total HpCDF	34	73	94	67	40	380	51	49
Total NpCDD	18	29	37	54	36	380	38	56
OCDF	10	52	50	23	25	180	41	33
OCDD	12	19	31	14	20	130	27	52
TCDD equivalents	9.1	10	11	11	10	53	12	5.6

*Source: Marklund et al., 1986a

However, in experiments in which known precursors were either left out or added to incinerator material, results were contrasting. Olie et al. (1983), comparing the emission from burned PCP-treated wood and 60-year-old painted untreated wood, detected no significant difference in PCDF (0.2 µg/g) and in PCDD formed. Hexachlorobenzene added in the proportion of 400 g to 2 m^3 municipal raw waste raised PCDF and PCDD emission (Olie et al., 1983). Chiu et al. (1983) burned PCB-treated wooden boxes and discovered PCDD congeners in the range of 0.4 µg/g and only OCDF at 0.025 µg/g. On burning pine wood with HCl, Tiernan et al. (1983) detected total PCDF (3 µg/g) that was 20 times more abundant than PCDD, while no PCDF formation was found on the combustion of untreated wood. Conversely, Nestrick and Lamparski (1982) detected PCDF and PCDD in soot and ash of incinerated untreated wood. Therefore, a third mechanism has been postulated in which PCDF and PCDD are formed *de novo* from nonchlorinated organic compounds in the presence of chlorine donors (Choudhry et al., 1982). Marklund et al. (1986b) have discussed the striking similarities in congener profiles of PCDFs and PCDDs found in emissions from municipal incinerators and pyrolysis of PVC, dichloroethane and hexachloroethane. They postulate a common mode of formation.

4.4.3. Sewage Sludge Combustion. Few data have been published on the production of PCDF from combustion of sewage sludges. However, sludges have been found to contain much synthetic chlorinated compounds so they could be sources of PCDF contamination. Lamparski et al. (1984) has reported PCDD in dried sewage sludge samples. Surprisingly small differences have been found between samples collected in 1981 and 1982 and dried samples collected in 1933 and sealed in glass until analysis. More recently, Weerasinghe et al. (1985a) analyzed samples of sewage sludge from two towns for PCDD and PCDF.

They detected several congeners of PCDD at both and, except for OCDF (1300 ng/g in one sample), no congeners of PCDF.

4.4.4. Electrical Equipment Fires. PCBs are used as dielectric fluid in two types of equipment: transformers containing mixtures of PCB and chlorobenzenes (PCBZ) and capacitors containing only PCB. Accidental fires involving transformers have been reported to produce PCDF and PCDD; when capacitors were involved only PCDF were formed.

A summary of PCDFs found after PCB fires (Rappe, 1983a,b,c, 1985a,b,c; Milby et al., 1985; Erickson et al., 1985; Williams et al., 1985; Hutzinger et al., 1985c) is provided in Table 4-21. The toxic 2,3,7,8-TCDF constitutes ~20-25% of the TCDF content, except for the Binghamton fire where it constituted 50% (Rappe et al., 1983a). The Binghamton fire concerned a dielectric fluid that contained 65% PCB and 35% chlorinated benzenes. This may explain why the Binghamton data are anomalous. The 2,3,4,7,8-PeCDF and 1,2,3,7,8/1,2,3,4,8-PeCDF were 18 and 9% of the total PeCDF, respectively (Rappe et al., 1983a). In Binghamton soot the 1,2,3,7,8- and 2,3,4,7,8-PeCDF, and 1,2,3,4,7,8-, 1,2,3,6,7,8- and 2,3,4,6,7,8-HxCDF were 46, 7.2, 32, 16 and 10%, respectively, of their isomeric class.

4.4.5. Incineration of Hazardous Waste. PCDFs have been reported in emission of hazardous waste incinerators (Tiernan et al., 1983; Rappe et al., 1983a). A Swedish waste incinerator was monitored weekly for PCDF and PCDD emissions that were strongly correlated with the levels of total chlorine input (Oberg et al., 1984). Table 4-18 contains other examples of PCDFs produced during the incineration of hazardous wastes.

TABLE 4-21

PCDFs Formed During PCB Fires

Site	Sample	PCDF Congener[a]						Reference
		Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Stockholm, Sweden, 1978	Liquid on capacitor	3-7[b]	2[b]	NM	NM	NM	NM	Jansson and Sundstrom, 1982
	PCB after explosion	11[b]	3[b]	NM	NM	NM	NM	
Stockholm, Sweden, 1981	Floor wipe	NM	1600	175	<0.5	<1	<1	Rappe et al., 1982, 1983a, 1985b
Binghamton, NY, 1981	Floor soot	NM	0.028	0.670	0.965	0.460	0.040	Rappe et al., 1983a O'Keefe et al., 1985a Schecter and Tiernan, 1985
	Ceiling soot	NM	162	197	110	36	14	
	Floor soot	NM	100	120	70	20	4	
	Air	NM	4-195[b]	ND-60[b]	2-9[b]	NM	NM	
	Air	NM	150[b]	50[b]	2-3[b]	NM	NM	
Surahammar, Sweden, 1982	Room floor							
	Wipe 1	NM	4000	3300	1800	1500	300	Rappe et al., 1985b,c
	Wipe 2	NM	90	25	17	17	4	
	Wall wipe 1	NM	1250	355	150	65	13	
	Wall wipe 2	NM	480	210	140	60	30	
Imatra, Finland, 1982	Soot 1	NM	1	0.2	0.04	0.02	0.01	Rappe et al., 1985b Elo et al., 1985
	Soot 2	NM	16	1.0	0.3	0.2	0.1	
	Soot 3	200	20	1	NM	NM	NM	
Hallstahammar, Sweden, 1982	Metal bar							
	Wipe 1	NM	1600	360	600	800	1340	Rappe et al., 1985b,c
	Wipe 2	NM	5000	1800	850	550	440	
	Floor wipe	NM	50	12	10	30	20	
Railway locomotive, Sweden, 1982	Floor wipe 1	NM	6900	29,000	14,000	6200	140	Rappe et al., 1985b,c
	Floor wipe 2	NM	4900	1500	400	170	40	
Kisa, Sweden, 1983	Floor wipe 1	NM	100,000	NM	NM	NM	NM	Rappe et al., 1985b
	Floor wipe 2	NM	500	NM	NM	NM	NM	

TABLE 4-21 (cont.)

Site	Sample	PCDF Congener[a]						Reference
		Cl_3	Cl_4	Cl_5	Cl_6	Cl_7	Cl_8	
Skovde, Sweden, 1982	Floor wipe 1	NM	100	40	40	8	5	Rappe et al., 1982
	Floor wipe 2	NM	600	100	60	8	5	Rappe et al., 1983a
	Wall wipe	NM	<1	<1	<1	<1	<1	Rappe et al., 1985b
	Bench wipe	NM	<1	10	<1	<1	<1	
London, U.K., 1983	Overheated PCB	NM	50-90	460	530-590	NM	ND	Cull and Jacobs, 1984
	Fresh PCB	NM	ND-70	150	130-160	NM	ND	
Turin, Italy	Concrete floor wipe	NM	3852	296	<9.5	<10	<0.5	Tumiatti et al., 1986

[a] μg/g unless otherwise stated

[b] pg/m^3

NM = Not mentioned

ND = Not detected

4.4.6. Car Exhaust. Ballschmiter et al. (1986) identified a series of PCDFs and PCDDs in used motor oil from automobiles. The congener profile was similar to that from incinerators burning urban waste. It was suggested that chlorinated additives in the oil were involved. Marklund et al. (1986b) found that the appearance of PCDFs/PCDDs depended on the presence of 1,2-dichloroethane scavenger. Contaminants added to the motor oil were not responsible. The average emission from a car running on leaded gasoline was 30-540 pg 2,3,7,8-TCDD equivalents/km, equivalent to 10-100 g 2,3,7,8-TCDD equivalents/year.

4.4.7. Other Sources. PCDFs have also been found in the emissions from steel mills and copper mills, baths using recycled material contaminated by PVC and polychlorinated paraffins (Marklund et al., 1986a). Bergman et al. (1984) also reported on the formation of PCDFs from chlorinated paraffins. PCDFs have been mentioned in several patents for use in electrical insulation (British Thompson-Houston Co. Ltd., 1939), in capacitors (Clark, 1940), flame retardants (Dazzi et al., 1974; Boyer, 1971) and as bactericides (Shibata et al., 1952). Commercial usage of the products is not available.

5. ENVIRONMENTAL FATE, TRANSPORT AND DISTRIBUTION

5.1. SUMMARY

PCDFs are ubiquitous contaminants. They are associated with the small particulate fraction and vapor of flue gas and can thus be transported in the atmosphere over very wide areas. Weather conditions can greatly affect their transport. Photodegradation by sunlight may occur in the presence of triplet sensitizers in aqueous solution; photodecomposition in the vapor phase has not been investigated.

In aquatic media, since PCDFs have poor solubility and tend to be adsorbed, they are expected to be persistent in sediments or on particulate matter. Their presence has been associated with atmospherically transported waste incineration-derived PCDF. In soil they have been detected in top soil layers after improper disposal of wastes or after dissemination of herbicides, but quantitative data are lacking. As for translocation potential from soil to vegetation is concerned, PCDFs tend to be adsorbed on external surfaces. Little data on bioconcentration factors exist.

5.2. INTRODUCTION

Since PCDFs are trace impurities in various chlorinated chemicals (see Section 4), they will accompany these chlorinated chemicals when they are released into the environment. As concentrations are generally below ppm levels, the quantity released determines the extent of the pollution. The content of toxic isomers also determines whether toxic effects may occur.

PCDFs have also been identified in effluents from various combustion processes (Section 4). Municipal solid wastes and industrial waste incinerations have been found to emit fly ash and flue gas containing PCDF in the range of $\mu g/m^3$. As PCDF may be associated with the small particulates and with flue gas, PCDFs could be distributed over large areas. This combustion may be responsible for the ubiquitous PCDF pollution of the environment.

Very little data for the PCDFs are available, so they are expected to act like PCDDs in the environment. The U.S. EPA (1985) gives an excellent summary of the environmental fate, transport and distribution of 2,3,7,8-TCDD.

5.3. ENVIRONMENTAL TRANSPORT

5.3.1. Air.

It is very difficult to assess the quantity and the isomeric distribution of PCDFs in the atmosphere as many variables interfere. Emission from incinerators is dependent on the refuse, combustion design, operating conditions, composition of the fuel, operating temperatures and degree of emission control. The great heights of stacks contribute to disperse PCDF over large areas and reduce concentrations near the source. Weather conditions, height of the stack in relation to the size and position of natural obstructions or buildings, relative distribution of PCDFs in flue gas between particulate fraction and particle-free gas phase (see 4.4.2.1.), and particle size are all factors that greatly modify the transport of PCDF in the atmosphere, as has been suggested for the other pollutants (Somers, 1971).

PCDFs may theoretically undergo photodecomposition in the atmosphere, but the environmental relevance of this process is not known. Photochemical degradation has been demonstrated under laboratory conditions for the highest chlorinated congeners, but these reactions are unlikely to occur in sunlight (Section 4.3.). Thus PCDFs are probably carried directly to the soil or to water compartments such as lakes and oceans.

A method to collect PCDFs and PCDDs in both the particulate and vapor phase is now available (Oehme et al., 1986). This method involves a fiber glass filter and a polyurethane foam plug from which the PCDFs were desorbed by Soxhlet extraction with toluene, followed by liquid chromatographic separation, and GC/MS confirmation and quantitation. The recovery from the filter is ~75-85% for $^{13}C_{12}$-1,2,3,4-TCDD, $^{37}Cl_{8}$-OCDD, and $^{13}C_{12}$-PeCDF; the recoveries from the foam are >80%. Typically, the PCDF congeners below HxCDF were found on the foam plug following the filter, while the rest were found adsorbed to particles on the filter. As soot loading increased, more of the lower congeners were found on the filter adsorbed to soot particles. Samples near an industrial city (Essen, Federal Republic of Germany) reflected the congener patterns of nearby municipal incinerator stack emission patterns. Concentrations decreased with distance away from the source. Samples in suburbia and in remote areas contained mostly PCDFs. Of the 60 samples taken, the highest levels of 5-10 pg PCDDs +PCDFs/m^{3} were found in a heavily industrialized area. In suburban areas the levels were 5-10 times lower. Thus people living near industrial areas may be exposed. The highest 2,3,7,8-TCDD equivalent level found was 0.1 pg/m^{3} with 2,3,7,8-TCDD always below detection. Ballschmiter et al. (1986) have shown that PCDFs can be emitted from leaded automobile exhaust but not from unleaded exhaust (Chapter 4 also has a discussion of this topic from the perspective of accumulation of PCDDs and PCDFs in leaded gasoline through use. The 1,2,-dichloroethane additive was postulated to be the chlorine donor). Ballschmiter et al. (1986) showed through pattern recognition that both automobile exhaust and emissions from municipal incinerators were correlated with lake sediments around the world. The municipal incinerator component was postulated to arise mostly from pentachlorophenol incineration (Cruczwa and Hites, 1984, 1985, 1986; Cruczwa et al., 1984, 1985). The striking uniformity of the patterns of isomers in a given congener class suggested a more global source of PCDFs/PCDDs like automobile exhaust. Three basic emission sources were postulated (Ballschmiter et al., 1985) based on congener profiles.

5.3.2. Water.

PCDFs are lipophilic and practically insoluble in water (Section 2.3.3.). Thus in aquatic media PCDF are present in the adsorbed state to particulate matter rich in organic content. Rappe et al. (1984) found concentrations (100 ng/ml) of PCDF in a suspension of soot/dust in wash water from a PCB fire to be only in the adsorbed state, since when the soot settled the water did not contain detectable PCDF. Abiotic degradation (photoreactions, hydrolysis, etc.) and biodegradation are not likely to occur in this compartment. Thus PCDFs are expected to be very persistent in aquatic media where they show high affinity for sediments. Czuczwa et al. (1984), Czuczwa and Hites (1984) and Czuczwa et al. (1985) found that fluxes of PCDF to lake sediments in the United States and Switzerland were ~0.1-0.3 ng/cm^{-2}/year^{-1}.

5.3.3. Soil.

PCDFs are rapidly and strongly adsorbed into most soils and sediments where they are expected to be immobile. Quantitative data on PCDFs are lacking, but studies on 2,3,7,8-TCDD indicate that these compounds are practically immobile (Helling et al., 1973), the apparent half-life for TCDD in soil exceeding 10 years (Di Domenico et al., 1980a). PCDF can be transported from soil to air or the water compartment through contaminated airborne dust particles or waterborne eroded soil.

Volatilization is probably not important because of the low vapor pressures of such compounds. However, Di Domenico et al., (1980b) suggested that volatilization could be at least partially responsible for the observed disappearance of TCDD from the topmost soil layers of the polluted Seveso area, 1 year after the accident. Freeman and Schroy (1985) modeled the volatilization of 2,3,7,8-TCDD adsorbed to Times Beach soil based on monitoring the volatilization over a period of 1 year. Thus it is likely that volatilization will occur also for the PCDFs, and this should be demonstrated.

In general, higher chlorinated PCDDs and PCDFs volatilize more slowly than lower chlorinated dioxins since their vapor pressures decrease with increasing chlorination (Table 2-5).

5.4. ENVIRONMENTAL TRANSFORMATION

5.4.1. Abiotic Transformation.

PCDFs are chemically quite stable and are not likely to be degraded by hydrolytic reactions under environmental conditions (Section 2.4.1.).

Photochemical degradation was discussed in Section 2.4.2. Photodegradation in organic solvents occurs for the higher PCDFs in organic solvents at 310 nm (Hutzinger et al., 1972a,b, 1973; Buser, 1976b; Firestone, 1977b; Mazer and Hileman, 1982; Mazer et al., 1983a,b,; Hileman et al., 1985). The degradation is much slower in water at 310 nm (Crosby and Moilanen, 1973).

Direct evidence of PCDF decomposition in the environment is lacking and the importance of sunlight in their environmental fate remains to be evaluated.

5.4.2. Biotransformation.

Currently virtually nothing is known about the biodegradation of PCDFs. However it is conceivable that they are relatively resistant to biodegradation, like the corresponding 2,3,7,8-TCDD (U.S. EPA, 1985). Only 5 of the 100 soil microbial strains tested have shown some ability to metabolize 2,3,7,8-TCDD (Matsumura and Benezet, 1973). Ward and Matsumura (1978) studied the biodegradation of 2,3,7,8-TCDD in Wisconsin lake waters and sediments and found a half-life of 550-590 days in sediments, while about 70% of 2,3,7,8-TCDD in lake water samples alone remained unaltered after 590 days. Moreover, Matsumura et al. (1983) estimated the half-life of 2,3,7,8-TCDD in an aquatic ecosystem model to be ~1 year. The occurrence of a polar metabolite of 2,3,7,8-TCDD with a probable hydroxy group in position 1 was found by Philippi et al. (1982) in several microbiological cultures after long incubation. Bumpus et al. (1985) demonstrated that ^{14}C-2,3,7,8-TCDD at a level of 14 pmol was biodegraded to the extent of ~25% by the white rot fungus, Phanerochaete chrysosporium. It was hypothesized that the extracellular lignin-degrading enzyme system was responsible. $^{14}CO_2$ was the metabolic product monitored. PCDFs are expected to cause the same behavior.

5.5. BIOACCUMULATION

High correlations have been observed between the octanol/water partition coefficient (K_{ow}) of many organic compounds and bioaccumulation in fish. Two equations have been proposed to relate the two measures. Lu et al. (1978) found that their data were fitted by the following equation:

$$\log [\text{bioaccumulation (fish)}] = 0.7 \log (\text{partition coefficient}) + 0.8$$

Therefore, high K_{ow} values would indicate high concentration factors. K_{ow} values were reported in Table 2-4. Using the Burkhard and Kuehl

(1986) recalculated values, the values of the log [bioaccumulation (fish)] defined by Lu et al. (1978) would be 4.51, 4.87 and 6.95 indicating high bioaccumulation for 2,8-DCDF, 2,3,7,8-TCDF and OCDF, respectively. The value for the 2,3,7,8-TCDF agrees with that for 2,3,7,8-TCDD (Burkhard and Kuehl, 1986). PCDFs have not been studied in model ecosystem.

6. ENVIRONMENTAL LEVELS AND EXPOSURE

6.1. SUMMARY

PCDFs have been found in wastewaters and rivers, and also in the fat of animals, mostly as acutely toxic 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, respectively. Plants do not appear to bioaccumulate PCDFs. The similarity of lake sediments with respect to PCDF and PCDD congeneric patterns implies that there may be a common source of PCDFs and PCDDs with air as the transport medium. Both the emissions from municipal incinerators and from leaded automobile exhausts are correlated to PCDFs and PCDDs. This does not support Dow's trace chemistries of fire hypothesis. PCDFs in milk and from industrial accidents are important routes of exposure also.

6.2. ENVIRONMENTAL LEVELS

6.2.1. Introduction. PCDFs have not been monitored systematically in the environment. Zitko (1972) and Zitko et al. (1972) investigated the presence of PCDFs in several wildlife samples with negative results, but the analytical methodology used in these studies had a sensitivity several orders of magnitude less than that required for detection of the probable environmental concentrations (ppt) of PCDFs. In a similar study, Bowes et al. (1973) investigated the presence of PCDFs in wildlife populations with high rates of embryonic death. Again, no PCDFs were identified, either in herring gull egg samples containing a total of ~0.9 g PCBs or a sea lion sample containing ~1 mg of PCBs.

The first positive report concerning the presence of PCDFs in the environment was by Jungclaus et al. (1978) who investigated the presence of anthropogenic organic chemicals in the wastewater of a chemical manufacturing plant and using GC/MS identified a TrCDF among >100 compounds. The same compound was identified in the water of a river receiving the industrial wastewater.

Table 6-1 contains a summary of the levels found in environmental media to 1986. PCDFs have been found in wastewater, river water, sediments and air samples.

6.2.2. In Soil and Sediments. PCDFs deposited in soil strongly adhere to the upper soil layers. Thibodeaux (1983) stated that PCDFs and PCDDs applied to the surface of soils having a high organic content generally stay in the upper 6-12" layer, while in more sandy soils they can migrate 3 feet or more. However sediments seem to be the ultimate sinks of PCDFs. Czuczwa et al. (1984, 1985) studied the presence of PCDFs and PCDDs in sediments and sediment cores from the Great Lakes in the United States (Czuczwa and Hites, 1984, 1985, 1986), and annually in laminated sediments from lakes in Switzerland (Czuczwa, et al. 1985). The accumulation of PCDDs and PCDFs paralleled the rapid growth of the production of chlorinated chemicals since 1940. In fact PCDFs and PCDDs were absent from sediments before 1945, but increased thereafter. In all cases OCDD predominated (>1000 ppt); HpCDD and HpCDF were the next most abundant congeners (1-15 ppt). The congener profiles from Switzerland and from U.S. sediments were similar.

The absence of PCDFs from the sediments before 1940 indicates that wood burning, coal combustion and probably leaded automobile emissions, which were prevalent before 1940, are not important sources of PCDFs. The similarity in the PCDF/PCDD profiles from the sediments in Switzerland and the United Stages suggests a common source involving atmospherically-transported combustionderived PCDFs (Chapter 5). The PCDDs and PCDFs in the sediments from Siskiwit Lake located in Isle Royale in Northern Lake Superior could only have been deposited from the atmosphere (Czuczwa et al., 1984). The congener profile of PCDFs and PCDDs in sediments was similar to that in airborne particulate samples from two locations remote from Siskiwit Lake.

TABLE 6-1

Levels of PCDFs in Environmental Media

Site	Sample	TCDF Concentration	Reference
Water	Wastewater, U.S. Riverwater, U.S.	Trace of a TrCDF Trace of a TrCDF	Jungclaus et al., 1978
	Narragansett Bay, RI, Water and suspended particles	2,4,8-TrCDF found; 0.013-0.25 ng/mℓ	Lake et al., 1981
Soil/Sediments	Hudson River Sediment, U.S.	Cl_4, <3-200; Cl_5, <3-193; Cl_6, <3-377; Cl_7, <3-2436; Cl_8, <3-1010 pg/g	Petty et al., 1983a
	Woods Pond Sediment, MA	Cl_4, <5; Cl_5, 9; Cl_6, 150; Cl_7, 920; Cl_8, 270 pg/g	
	Saginaw River/Lake Huron:		
	Surface Sediments (6)	Cl_4, 0.1-3; Cl_5, 0.01-4; Cl_6, 0.13-12; Cl_7, 0.2-30; Cl_8, 0.05-7 ng/g	Czoczwa and Hites, 1984, 1986
	Sediment Cores to 12 cm	Cl_4, 10-30; Cl_5, 100-200; Cl_6, 100-200; Cl_7, 50-400; Cl_8, 5-10 pg/g	
	Buffalo River Sediment, IL	2 TCDFs, OCDF detected	Kuehl et al., 1984b
	Sediments:		
	Albany, Hudson River, NY	5 ng/g 2,3,7,8-TCDF	O'Keefe et al., 1984
	Tappan Zee Bridge, Hudson River, NY	29-46 ng/g 2,3,7,8-TCDF	
	Siskiwit Lake Sediment, MI		
	Cores 0-6 cm	Cl_4, Cl_5, 2-5; Cl_6, 2; Cl_7, 17-20; Cl_8, 3-4 pg/g	Czuczwa et al., 1984, 1985; Czuczwa and Hites, 1986
	8-9 cm	Cl_4, <0.4; Cl_5, <0.4; Cl_6, <0.4; Cl_7, <0.4-1.6; Cl_8, 1.1 pg/g	
	NBS Urban Dust	Cl_4, 0.03-0.10; Cl_5, 0.31-1.3; Cl_6, ND-1.6; Cl_7, ND-5.8; Cl_8, ND-1.9 ng/g	Li et al., 1985
	Surface Sediments from lakes in Switzerland	Cl_4, 13-170; Cl_5, <10; Cl_6, 5-60; Cl_7, 100-240; Cl_8, 10-510 ng/g	Czuczwa et al., 1985; Czuczwa and Hites, 1985
	Soil, Amsterdam, The Netherlands	Cl_4, 1413-24,000; Cl_5, ND-5650; Cl_6, ND-115 ng/g	Heida et al., 1985

TABLE 6-1 (cont.)

Site	Sample	TCDF Concentration	Reference
Soil/Sediments (cont.)	60 Vapor and particulate air samples, Federal Republic of Germany	5-10 pg/m^3 for sum of PCDDs and PCDFs	Oehme et al., 1986
	Surface sediments from:		
	Lake Michigan (2)	Cl_4, <10; Cl_5, <10; Cl_6, 8-33; Cl_7, 70-240; Cl_8, 0.5-24 ng/g	Czuczwa and Hites, 1986
	Lake Erie (2)	Cl_4, <10; Cl_5, <10; Cl_6, <10; Cl_7, 40-80; Cl_8, 30-60 ng/g	
	Lake Ontario	Cl_4, <10; Cl_5, <10; Cl_6, 200; Cl_7, 1200; Cl_8, 3600 ng/g	
Air	Air Particulate		
	Washington, DC (NBS)	Cl_4, 1.3; Cl_5, 1.2; Cl_6, 0.8; Cl_7, 18; Cl_8, 6.2 ppb	Czuczwa et al., 1984, 1985; Czuczwa and Hites, 1986
	St Louis, MO (NBS)	Cl_4, 0.2; Cl_5, 0.2; Cl_6, 0.3; Cl_7, 12; Cl_8, 0.5 ppb	
	Air Sample, New York	<0.1-1.9 pg/m^3 2,3,7,8-TCDF	O'Keefe et al., 1985b
	U.S. Incinerator Plant Air	Total PCDF was 3 ng/m^3	Haile et al., 1985

ND = Not detected

They showed that the pattern of PCDDs/PCDFs in lake sediments resembled that of the contaminants in chlorophenols and could be detected in sediments to a depth of 12 cm. This does not support Dow's trace chemistry of fires hypothesis. Reviews have been written by Hutzinger et al. (1985a,b) and Weerasinghe and Gross (1985). A mathematical model for the transport/fate of organics in soils has been published by Lindstrom and Piver (1985).

6.2.3. In Plants. Few data on the concentrations of PCDFs in plants and vegetables exist. Wild fruits collected from a Dutch area seriously contaminated with organochlorine waste compounds showed no presence of PCDFs and PCDDs (Table 6-2) which, however, were present in topsoil, in fish and in animals (Heida and Olie, 1985). Since there is little bioaccumulation of PCDDs in plants, probably PCDFs will not bioaccumulate to any extent either (U.S. EPA, 1985). Plants' aerial parts may be contaminated through deposition of airborne particles or through volatilization or translocation of the compounds present in soil. When 2,3,7,8-TCDD was added to soil, only 0.15% accumulated in oats and soya beans (Isensee and Jones, 1971). Aerial portions of plants growing in contaminated Seveso areas contained less 2,3,7,8-TCDD than roots, which had lower levels than the surrounding soil. At a soil concentration of 10,000 ppt, fruits were found to contain no more than 37 ppt, with 95% 2,3,7,8-TCDD being located in the peel. This strongly suggests that contamination was due to surface dust deposition and not to plant uptake (Wipf et al., 1982). Underground tissues of some plants such as carrots can take up lipophilic chemicals from the soil (Cocucci et al., 1979; Pocchiari et al., 1983). The 2,3,7,8-TCDD concentration in root vegetables ranged from 3-10%, and in the aerial parts from 0.3-1% of the surrounding highly contaminated soil (Pocchiari et al., 1983).

TABLE 6-2

Ranges of 2,3,7,8-TCDD and PCDFs in Top Soil and Biological Samples in ppt fresh weight in a Dutch Hazardous Waste Dump[a]

Material	Number of Samples Analyzed	2,3,7,8-TCDD	Total TCDFs	Total PeCDFs	Total HxCDFs
Milk fat	6	<17	NA	NA	NA
Top soil (dry weight)	9	<20-1929	1413-23,920	ND-5650	ND-115
Worms	9	<15-889	ND-3020	ND-300	ND-2694
Mice/liver	4	<10-5237	ND-13809	1408-23,097	ND-584
/reference liver	1	<68	ND	ND	ND
Rabbits/liver	14	<10-50	ND-168	ND-1121	ND-1081
/reference liver	5	<2-<50	ND	ND-124	ND-132
Pheasants/liver	8	<6-33	ND	ND	ND
/reference liver	2	<15-<19	ND	ND	ND
Eel-inside dump/liver	2	96-97	2350-2765	ND-169	ND
/muscle and fat	2	104-144	4276-5193	182-558	ND
Eel-outside dump/liver	-	NA	NA	NA	NA
/muscle and fat	3	30-42	41-47	134-147	ND
/reference liver	1	<15	NA	NA	NA
Pike-inside dump/liver	1	584	959	975	ND
/muscle	1	21	41	154	ND
Pike-outside dump/liver	3	<50-103	ND	ND-1419	ND
/muscle	2	<30	ND	263-268	ND

TABLE 6-2 (cont.)

Material	Number of Samples Analyzed	2,3,7,8-TCDD	Total TCDFs	Total PeCDFs	Total HxCDFs
Pike-reference/liver	1	<9	ND	ND	ND
/muscle	2	<13-<14	ND	ND	ND
Minnow-inside dump/liver[b]	1	593	5808	ND	ND
Minnow-outside dump/liver	1	<20	ND	ND	ND
Blackberries	8	ND-<10	ND	ND	ND
Elderberries	2	<2-<17	ND	ND	ND
Pumpkin	1	<20	ND	ND	ND

[a] Source: Heida and Olie, 1985

[b] Only one specimen analyzed

ND = Not detectable

NA = Not analyzed

6.2.4. **In Aquatic Organisms.** Evidence for aquatic wildlife contamination by PCDFs was reported by Stalling et al. (1981), Kuehl et al. (1981) and Rappe et al. (1981). Samples of fat from snapping turtles, (*Chelyda serpentina*), from the Hudson River and Baltic gray seal, (*Halichoerus grupus*), and from the Gulf of Bothnia were examined using a direct-probe negative chemical ionization mass spectrometric technique of sensitivity 0.2 pg for 2,3,7,8-TCDF. The turtle fat containing >750 ppm of PCBs was found to be contaminated by PeCDFs and HxCDFs while the seal fat, whose concentration in PCBs was ~100 ppm, contained trace amounts of PeCDFs (Stalling et al., 1981; Rappe et al., 1981).

The same two samples were analyzed by Rappe et al. (1981) using high resolution GC/MS in order to identify the single PCDF isomers. The isomeric PCDF composition of the two samples showed a good correspondence despite the differences between the two species and the different origins. The turtle fat sample had a total PCDF concentration of 3 ppb distributed among 18 different isomers; among these the toxic 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF were present at a concentration of 45 and 620 ppt, respectively. HpCDFs, PeCDFs and HxCDFs predominated in that order (1000, 820 and 700 ppb, respectively).

In the seal fat, the total amount of PCDF was 40 ppt with the 2,3,7,8-TCDF at 1 ppt and the 2,3,4,7,8-PeCDF at 15 ppt. The composition of the major isomers found in these biological samples corresponded fairly well to the pattern present in commercial PCBs while a poor correlation was found with the isomer composition of other PCDF sources such as polychlorinated phenols and incinerator fly ash samples. This suggested that the presence of PCDFs in these samples was correlated with PCB pollution.

Yamagishi et al. (1981) also detected the presence of unspecified TCDFs in Japanese fish (Zacco platypus), and Kuehl (1981) reported that fish taken in U.S. rivers before 1978 contained a total estimated PCDFs ranging from 0.5-5 pg/g. Lake et al. (1981) quantitated a TrCDF in Rhode Island aquatic life. In 1978, the mussel (Mytilus edudis) contained 643 ppb and six samples in 1979 were found to have 52-1180 ppb. Clams (Mercenaria mercenia) also contained 26 ppb, whereas lobster (Homorus americanus) hepatocytes had 1260 ppb. Kuehl et al. (1986) also detected TrCDFs, TCDFs, PeCDFs, HxCDFs and HpCDFs in fish from the Great Lakes.

These discoveries ushered in many investigations (Table 6-3) involving the major isomeric PCDF classes in tissues of fish living in lakes, rivers and seas.

PCDFs were identified but not quantitated in fish from 18 major watersheds near the Great Lakes collected in 1979-1981 (Kuehl et al., 1984a). They have been identified in fish from the Great Lakes (O'Keefe et al., 1983; Ryan et al., 1983; Stalling et al., 1983). PCDDs were not detected in fish samples from Lake Superior while a series of PCDFs could be identified in the same samples, indicating more widespread background levels for the PCDFs than for PCDDs (Stalling et al., 1983). Stalling et al. (1983) could detect very little 2,3,7,8-TCDF in sediments but larger amounts in fish; OCDF was abundant in sediments but low in fish. Similar results (Table 6-4) were obtained in examining sediments and yellow perch from Woods Pond, which was known to be contaminated by Aroclor 1260 (Petty et al., 1983a,b). Stalling (1982) found carp, largemouth bass and striped bass from the lower Hudson River contained 2,3,7,8-TCDF at concentrations ranging from 14-18 pg/g. The presence of 2,3,7,8-TCDF in striped bass was confirmed by O'Keefe (1982). Since the upper section of the Hudson River was highly contaminated

TABLE 6-3

PCDF Residues in Wildlife

Wildlife	Species	PCDF Level (pg/g)	Reference
Animals	Fat from Snapping Turtle, U.S. (Chelyda serpintina)	Cl_4, 45; Cl_5, 820; Cl_6, 700; Cl_7, 1000; Cl_8, 350	Rappe et al., 1981; Stalling et al., 1982
	Fat from grey seal, Sweden (Halichoerus grupus)	Cl_4, 1; Cl_5, 15; Cl_6, 8; Cl_7, 10; Cl_8, 3	
	Seal, Sweden	Cl_4, 1; Cl_5, 16; Cl_6, 8; Cl_7, 10; Cl_8, 3	Buser et al., 1985
Fish	Yellow Perch, MA	Cl_4, 1.1; Cl_5, 0.64; Cl_6, 0.44; Cl_7, <0.005; Cl_8, <0.005	Petty et al., 1983a,b
	Bloater, Lake Superior	Cl_4, 26; Cl_5, 9; Cl_6, 2; Cl_7, 1; Cl_8, 2	Petty et al., 1983b; Stalling et al., 1983
	Lake Trout (Salvelinus namaycush from:		
	Lake Superior (2)	Cl_4, 10-19; Cl_5, 5; Cl_6, ND-5; Cl_7, ND-4; Cl_8, trace-36	
	Lake Ontario (2)	Cl_4, 34-40; Cl_5, 24-48; Cl_6, 10-29; Cl_7, ND-6; Cl_8, ND-2	
	Lake Michigan (3)	Cl_4, 19-35; Cl_5, 41-61; Cl_6, 8-16; Cl_7, 1-4; Cl_8, 1-6	
	Lake Huron	Cl_4, 19; Cl_5, 9; Cl_6, 242; Cl_7, <1; Cl_8, <1	
	Brook Trout, (Salvelinus tontinalis) in Lake Ontario	Cl_4, 19; Cl_5, 4; Cl_6, 3; Cl_7, 3; Cl_8, 3	Stalling et al., 1983
	Walleye (Stizostedion v. vitreum):		
	Lake Michigan	Cl_4, 4; Cl_5, 4; Cl_6, 0.7; Cl_7, 0.7; Cl_8, 3	Stalling et al., 1983
	Lake Erie	Cl_4, 18; Cl_5, 9; Cl_6, 6; Cl_7, 5; Cl_8, 2	
	Common carp (Ctenopharyngodon idella):		
	Lake Erie	Cl_4, 5; Cl_5, 5; Cl_6, 2; Cl_7, 4; Cl_8, 2	Stalling et al., 1983
	Lake Huron	Cl_4, 27; Cl_5, 44; Cl_6, 34; Cl_7, 44; Cl_8, 4	
	Lake Michigan (2)	Cl_4, 5-37; Cl_5, 12-73; Cl_6, 5-145; Cl_7, 6-31; Cl_8, 1-4	
	Rainbow Trout (Salmo gairdneri), Lake Ontario	Cl_4, 39; Cl_5, 10; Cl_6, 2; Cl_7, ND; Cl_8, ND	Stalling et al., 1983

TABLE 6-3 (cont.)

Wildlife	Species	PCDF Level (pg/g)	Reference
Fish (cont.)	Lake Ontario Fish:		
	Smelt (4)	3-112 Cl_4	Ryan et al., 1983
	Catfish	99 Cl_4	
	White Perch	15 Cl_4	
	Lake Trout (2)	8.5-24 Cl_4	
	Rainbow Trout	200 Cl_4	
	Ontario Salmon	153 Cl_4	
	Herring, Sweden	Cl_4, 50; Cl_5, 430; Cl_6, 420; Cl_7, 1400; Cl_8, 100	Buser et al., 1985
	Guillemot, Sweden	Cl_4, 10; Cl_5, 800; Cl_6, 1000; Cl_7, 2500; Cl_8, 250	
	Herring, Sweden	Cl_4, 5; Cl_5, 8; Cl_6, 2	Rappe et al., 1985d
	Herring, Sweden	Cl_4, 4; Cl_5, 8; Cl_6, 2	
	Guillemot, Sweden	Cl_4, 2; Cl_5, 184; Cl_6, 41	
Bird	Herring gull (Larus argentatus), Lake Huron	Cl_4, 15-16; Cl_5, 28-50; Cl_6, 40-57; Cl_7, 7-17; Cl_8, 3-5	Stalling et al., 1983
Ecosystem	Contaminated animals and fruits in a Netherlands Dump:		
	Worms (9)	Cl_4, ND-3020; Cl_5, ND-300; Cl_6, ND-2694	Heida and Olie, 1985
	Mouse liver (4)	Cl_4, ND-13,800; Cl_5, 1408-23,097; Cl_6, ND-584	
	Rabbit liver (14)	Cl_4, ND-168; Cl_5, ND-1121; Cl_6, ND-1081	
	Pheasant liver (8)	Cl_4, Cl_5, Cl_6, ND	
	Eel liver (2)	Cl_4, 2350-2765; Cl_5, ND-169; Cl_6, ND	
	Eel muscle/fat (2)	Cl_4, 4276-5193; Cl_5, 182-558; Cl_6, ND	
	Reference eel muscle/fat (3)	Cl_4, 41-47; Cl_5, 134-147; Cl_6, ND	
	Pike liver	Cl_4, 959; Cl_5, 975; Cl_6, ND	
	Pike muscle	Cl_4, 41; Cl_5, 154; Cl_6, ND	
	Pike liver 2 (3)	Cl_5, ND-1419; Cl_4, Cl_6, ND	
	Pike muscle 2 (2)	Cl_5, 263-268; Cl_4, Cl_6, ND	
	Minnow liver 1	Cl_4, 5808; Cl_5, Cl_6, ND	
	Minnow liver 2	Cl_4, Cl_5, Cl_6, ND	
	Blackberries (8)	Cl_4, Cl_5, Cl_6, ND	
	Elderberries (2)	Cl_4, Cl_5, Cl_6, ND	
	Pumpkin (1)	Cl_4, Cl_5, Cl_6, ND	

ND = Not detected

TABLE 6-4

PCDF Distribution in a Pond Known to be Contaminated by Aroclor 1260*

	PCDF Concentration (pg/g)		
	Sediment	Yellow perch	Aroclor 1260
TCDF	0.005	1.06	290
PeCDF	0.009	0.64	1330
HxCDF	0.15	0.44	1810
HpCDF	0.92	0.005	780
OCDF	0.27	0.005	29
Total PCDF	1.35	2.14	4240
PCB (μg/g)	60	170	neat
PCDF/PCB			
Ratio	22.5×10^{-6}	12.6×10^{-6}	4.24×10^{-6}

*Source: Petty et al., 1983a,b

by PCBs from industrial discharges (Horn et al., 1979), O'Keefe et al. (1984) compared the PCDD and PCDF concentrations in striped bass from this basin and from two other locations along the Atlantic coast. They found 2,3,7,8-TCDF in the fish from all three locations with concentrations varying from 6 ppt in Atlantic coast to 78 ppt in Hudson River. Analysis of sediments and of nonmigratory fishes confirmed the source of contamination.

Among fishes living inside and in the near surroundings of a dump contaminated with waste organochlorine compounds, eels accumulated the highest levels of PCDFs (see Tables 6-2 and 6-3). These amounted to 5,000 ppt in the whole body and 2500 ppt in the liver. The levels in topsoil were 1500-2400 ppt (Heida and Olie, 1985) (see Table 6-2).

A search for toxic 2,3,7,8-containing isomers in wildlife has also been conducted by many investigators (Table 6-5). As expected, the more highly chlorinated members bioaccumulated more than the TCDFs. The levels quoted are lower than the actual levels because recoveries varied widely between 50 and 100% (Smith et al., 1984). Stalling et al. (1985a,b) have also utilized principal components analysis to ascertain the sources of PCDFs in environmental media. The toxic 2,3,7,8-substituted isomers predominate in all wildlife with residues, these also being the predominant isomers found in Yusho victims. Norstrom et al., 91986) reported that crustaceans can contain isomers other than the 2,3,7,8-substituted.

Zitko and Choi (1973) and Zitko et al. (1973) provided the only available information concerning accumulation and metabolism of PCDFs in fish. Zitko and Choi (1973) fed a group of 35 juvenile Atlantic salmon (Salmo salar) several times daily for 140 days with dry fish food spiked with a mixture of 2.1, 4.4, 2.2 and 9.7 μg/g food, respectively. An analysis of the muscle and gut of fish that died between the 81st and 135th day and of fish that survived for 140 days indicated that only OCDF could be detected.

TABLE 6-5

Levels of Toxic PCDF Isomers in Wild Animals

Wildlife	Sampling Site/Species	PCDF (pg/g) 2,3,7,8- (Cl_4)	2,3,6,7- (Cl_4)	1,2,4,7,8-/ 1,3,4,7,8- (Cl_5)	1,2,3,7,8- (Cl_5)	2,3,4,7,8- (Cl_5)	1,2,3,4,7,8-/ 1,2,3,6,7,8- (Cl_6)	1,2,3,4,6,7,8- (Cl_7)	1,2,3,4,6,8,9- (Cl_7)	Reference
Animal	Hudson River, U.S./ Snapping turtle fat (Chelyda serpentina)	45				620				Rappe et al., 1981
	Gulf of Bothnia, Sweden/ Seal fat (Halichoerus grupus)	1				15				Rappe et al., 1981
	Seal fat	1			1	15	4	5	5	Buser et al., 1985
Fish	Lake Ontario, Canada									
	Smelt (4)	3.2-34								Ryan et al., 1983
	Catfish	54								
	White perch	15								
	Lake trout (2)	9-24								
	Rainbow trout	12								
	Ontario salmon	79								
	Pacific salmon	<10								
	Eel (2)	ND								
	Hudson River, Waterford, NY Carp (5), 1981	5								O'Keefe et al., 1984
	Goldfish (5), 1981	<9								O'Keefe et al., 1984
	Hudson River, Albany, NY Goldfish (3), 1981	<5								O'Keefe et al., 1984
	Hudson River, Tappan Zee Bridge									
	Striped bass (9), 1981	54								O'Keefe et al., 1984
	Striped bass (4), 1983	56								
	Hudson River, Poughkeepsie Striped bass (2), 1983	74-78								O'Keefe et al., 1984

TABLE 6-5 (cont.)

Wildlife	Sampling Site/Species	PCDF (pg/g)								Reference
		2,3,7,8- (Cl_4)	2,3,6,7- (Cl_4)	1,2,4,7,8-/ 1,3,4,7,8- (Cl_5)	1,2,3,7,8- (Cl_5)	2,3,4,7,8- (Cl_5)	1,2,3,4,7,8-/ 1,2,3,6,7,8- (Cl_6)	1,2,3,4,6,7,8- (Cl_7)	1,2,3,4,6,8,9- (Cl_7)	
Fish (cont.)	Bayonne Bridge, Newark Bay Striped bass, 1983	26								O'Keefe et al., 1984
	Chesapeake Bay, MD Striped bass (4), 1983	8.5								O'Keefe et al., 1984
	Long Island, NY Striped bass (4), 1983	16								O'Keefe et al., 1984
	Rhode Island Coast (4), 1983	25								O'Keefe et al., 1984
	Striped bass (5), 1982	14								
	Common carp 1	11	2	2	5	11	5	3		Stalling et al., 1985b
	2	11	3	3	1	4	2	<4		
	Grass carp	1.5		2	1	1	2	2		Stalling et al., 1985b
	Sweden									
	Herring	50		100	80	250	20	900	500	Buser et al., 1985
	Guillemot	10			50	750	150	1000	1500	
	Herring	4	1	1	1	6	1			Rappe et al., 1985d
	Herring	3	1	1	1	6	1			
	Guillemot	2	trace		4	180	34			
Bird	Hudson River, Catskill, NY									O'Keefe et al., 1984
	Black duck (6), 1980	79								
	Mallard duck, (12), 1980	44								
	Wood duck, 1980	13								
	Green Bay and Lake Michigan, WI Night heron (Nycticorax nycticorax)									Stalling et al., 1985a
	1978	<2-8								
	1982	4								
	Cormorant (Phalacrocorax aurith) 1983	2								Stalling et al., 1985a
	Herring Gull 1	4		<2	<2	16	7	<4		Stalling et al., 1985b
	2	2		<2	<2	20	4	<4		

The detection limit was ~0.02 µg/g wet weight for PCDF, TrCDF and TCDF. The concentration of OCDF in muscle was 0.03 µg/g in dead fish and 0.01 µg/g in surviving fish. The concentrations in gut were 0.21 µg/g in dead fish and 0.02 µg/g in living fish. The percent lipid in muscle and gut of dead and surviving fish was also measured and found to be higher in gut. Concentrations of OCDF in lipid were calculated to be 3.53 µg/g and 1.30 µg/g in muscle of dead and alive fish, respectively, and 17.36 and 0.99 µg/g, respectively, in gut of dead and live fish. Although the concentration of OCDF was higher in dying fish than in surviving fish, it is unknown whether OCDF caused the toxic effects. The data indicate, however, that the concentration of OCDF in fish was considerably lower than in the spiked food.

Zitko et al. (1973) also showed that only a small portion of orally administered 2,8-DCDF was accumulated in the tissues of brook trout (Salvelinus fontinalis). Several fish were fed a cumulative dose (in gelatin capsules) of 107-361 µg DCDF/g wet fish weight over variable exposure periods. The tissue residues ranged between 0.052 and 0.340 µg/g (0.01-0.13% of administered dose) in muscle and between 0.146 and 1.04 µg/g (0.08-0.4% of administered dose) in liver. Mass spectral analysis of the organic compounds isolated from the water containing excreta from one of the fish indicated the presence of a DCDF and of a hydroxy-DCDF metabolite.

The results of Zitko et al. (1973) and Zitko and Choi (1973) indicate that PCDFs are poorly absorbed in the gut of fish. The limited metabolic information provided by Zitko et al. (1973) suggests that DCDFs are hydroxylated by aquatic organisms.

6.2.5. In Birds. Tables 6-3 and 6-5 summarize the present data. O'Keefe et al. (1984) determined the presence of PCDF in three duck species collected near the Hudson River. Concentrations of 2,3,7,8-TCDF ranged from 13-79 ppt in fat tissues. PCDFs and PCDDs were also detected in birds and bird eggs in Wisconsin (Stalling et al., 1985a). 2,3,7,8-Chlorinated congeners were the major constituents. The pattern of PCDF and PCDD contamination was compared in birds and fishes living in the same Great Lakes area. The ratio of PCDFs to PCDDs in bird eggs were lower than in fish of the Great Lakes, indicating that in birds the rate of metabolism of PCDF is probably higher than that of PCDDs.

6.2.6. In Terrestrial Animals. These data are summarized in Tables 6-3 and 6-5. TCDFs were determined in tissues of a horse grazing close to a wire reclamation incinerator; TCDF (unspecified isomers) concentrations were 165 pg/g in the fat and 57 pg/g in the liver with unspecified TCDD levels of 45 and <6 pg/g in the fat and liver, respectively (Hryhorczuk et al., 1981). Recently Rappe et al. (1985) found 2,3,7,8-chlorinated PCDFs and PCDDs in milk from cows that had grazed near an incinerator in Switzerland. The levels were found to be related to the distance between the grazing area and incinerator.

Heida and Olie (1985) analyzed soil and biological samples collected from a household waste dump near Amsterdam, which appeared to be seriously contaminated with organochlorine compounds (2,4,5-T, dichlorobenil, lindane, tetrachlorodifon). Fish, game animals, worms and mice contained measurable amounts of the most toxic PCDDs and PCDFs (see Tables 6-2 and 6-3). The highest levels were detected in fish, especially eel (see Table 6-2). The worms tended to have the same congeneric pattern as soil (see Table 6-3), while in mice the toxic isomers prevailed probably as a result of differential biodegradation and excretion rates.

Recently, Ryan et al. (1985a) found PCDDs and PCDFs in 40 chicken and pork tissue samples also positive for pentachlorophenol. HxCDDs, HpCDDs and OCDDs were present in 50, 62 and 46% of the samples at mean levels of 27, 52 and 90 ppt, respectively. Similar levels of HxCDF and HpCDF were found in some of these samples. TCDFs, PeCDFs and OCDF were not detected. Comparison of the congeneric patterns of the chicken samples with those of pentachlorophenol-treated wood indicated that pentachlorophenol was probably the main source of contamination. Residues in humans are considered in Chapter 7.

6.3. EXPOSURE

6.3.1. Food. Food may be an important route of exposure since PCDFs have high bioaccumulation coefficients (Section 5.5.). Since their concentrations in PCBs is of most concern, it is instructive to calculate the maximum PCDF exposure levels associated with PCB exposure.

The present dietary intake in the United States of PCBs from fish is ~175 μg/day (Cordle et al., 1978). If the amount of 2,3,7,8-TCDF is the same as in a used Kanechlor 400 formulation (similar to Aroclor 1248), i.e., 1.25 ppm, the amount of this isomer stored if there is no excretion would be ~0.2 ng/day.

Workplace air levels of Aroclor 1242 can range as high as 2.22 mg/m^3 (Ouw et al., 1976). If it is present as airborne particulate, it would contain ~3 ng/m^3 of the 2,3,7,8-TCDF. If the same assumptions are used to estimate the levels of this isomer in fish, the levels of PCBs reported for various environmental compartments are as follows: ambient air (<100 ng/m^3), surface waters (10-50 ng/ℓ), foundry effluent (12-335 ppb), paper mill effluents (0.01-25 ppb), snow (0.17-0.24 ppb), wastewater (<520 ng/ℓ) and sewage sludge (16 mg/kg) (Chapter 4). PCB residues in human adipose tissues in the United States are near detection limits: below detection limit (34.2%), <1 ppm (33.3%), 1-2 ppm (27.3%) and >5.2 ppm (5.2%) (Yobs, 1972). Human milk can contain up to 100 ppb of PCBs (Savage et al., 1973). HxCDFs, HpCDFs and OCDFs have been detected in cow's milk, blood and tissue fat, after pentachlorophenol was ingested in the feed by a cow (Firestone et al., 1979). Thus cow's milk may be a source of exposure to animals exposed to PCDFs/PCDDs. Nygren et al. (1986) also reported low ppt levels of PCDFs and PCDDs in samples of bovine fat, bovine milk, and in commercial cream from Sweden and Scotland. Rappe et al. (1985e) also documented the PCDFs and PCDDs in bovine milk from Switzerland, with the highest levels being found in samples collected in proximity to municipal and other incinerators. Human milk is also another possible source (Chapter 7).

Concern has been expressed over the formation of PCDDs during the cooking of food. Liver, steak and hamburger from female Holsteins have been examined, but only raw liver contained significant amounts of PCDDs; levels decreased when the liver was cooked. Variation from sample to sample was high so that no firm conclusions could be reached (Zabik and Zabik, 1980). The situation is rather different for the PCDFs, especially if the tissues contain PCBs. The PCDFs might possibly be formed from PCBs during cooking, by the mechanisms cited in Section 2.5.2.1., and verification of this is important. Cooking appears to decrease PCB levels in foods such as poultry and milk as well as PBB levels in poultry (Zabik et al., 1978). The decline appears to be related to the amount of fat rendered in the cooking process, although a complete mass balance should be made using GC/MS. Firestone (1977b) found 0.4 ppb of HpCDFs and 0.4 ppb of OCDFs in 2 of 15 commercial U.S. gelatins. PeCDFs, HxCDFs and HpCDFs were found in one Mexican sample.

It was thought that the PCDDs/PCDFs in fly ash would not be bioavailable on ingestion (Hutzinger et al., 1985b), but rats, guinea pigs and hamsters fed fly ash admixed with their food showed 2,3,7,8-substituted isomers in their livers (Berg et al., 1985a). These results imply that fly ash-contaminated food, or particles in the air whose size is greater than respirable (15 μm) and which are cleared eventually to the stomach, will be partially absorbed by animals and probably humans.

6.3.2. Air. The inhalation of fly ash particles containing PCDDs and PCDFs is likely around municipal incinerators in addition to PCDFs emitted in automobile exhaust (Ballschmiter et al., 1986). PCDFs and PCDDs in both vapor and particulate form have been detected in Germany (Oehme et al., 1986). The vapor and respirable particles will be absorbed through the lung alveoli, and the nonrespirable particulate fraction may be partially ingested.

Another source of PCDF exposure is during and after electrical fires where PCBs were originally present (Chapter 4). Risk of exposure to PCDFs is high for fireman fighting electrical fires and the extent of exposures should be investigated as should workplaces involving PCBs including the following: chemical plant handling or manufacturing halogenated aromatics; factories making or repairing transformers or capacitors, using casting waxes or having heat-exchange systems; in offices utilizing carbonless copy paper containing PCBs; and during incineration of halogenated aromatics.

6.3.3. Other Exposure Routes. Analysis of latex nipples (Gorski, 1981) revealed PCDDs and PCDFs to be present. TCDFs and PeCDFs were not found, but HxCDFs (0.02-0.8 ppb), HpCDFs (0.1-3.1 ppb) and OCDFs (0.3-28 ppb) were confirmed. These were not extractable by water, unlike pentachlorophenol.

Gamma-irradiation of the nipples for sterilization purposes was suggested as a possible way that toxic isomers could be produced. Exposure through drinking water is unlikely.

7. TOXICOLOGICAL EFFECTS IN MAN AND EXPERIMENTAL ANIMALS

7.1. SUMMARY OF EFFECTS OF POLYCHLORINATED DIBENZOFURANS

7.1.1. Toxicokinetics. PCDFs are easily absorbed by passive diffusion across cell membranes. For the highest chlorinated congeners lipophilicity can result in such insolubility that absorption may be strongly impeded. The congeners with 2,3,7,8 chlorine-substitution are retained in tissues of animals and humans. The presence of two adjacent unsubstituted carbon atoms predispose towards metabolism and hence excretion; therefore these congeners have not been detected in the tissues of animals and humans even when exposed to high levels. Broad species variations have been observed in metabolism and excretion; the position that humans occupy regarding metabolic fate and excretion relative to other species is still undetermined. Physiological pharmacokinetic models developed in some animal species do not permit extrapolation to man since little metabolic information about the pattern of the different isomers within species is available. Some of the most relevant features are summarized here.

Studies on the absorption, metabolism and elimination of labeled 2,3,7,7-TCDF and of a mixture of PCDFs consisting of tetra-, penta- and hexa-CDFs in laboratory animals (rats, guinea pigs, monkeys, mice) show that CDFs preferentially accumulate in the liver and fat. The 2,3,4,7,8-PeCDF accumulated more than other isomers in the livers of monkeys and rats dosed repeatedly with the mixture. Accumulation of the same isomer several years after accidental contamination was also observed in the liver of human patients with Yusho or Yu-Cheng disease. Accumulation of PCDFs across the placenta in the rat fetus is limited, but transfer to suckling offspring is much greater. In the rat, the whole-body half-life of 2,3,7,8-TCDF is <2 days after a single i.v. dose of 30.6 μg/kg bw. In contrast, the guinea pig has a minimum whole-body half-life of 20 days after a single i.v. dose of 6 μg/kg. A maximum whole-body half-life of 40 days was calculated in guinea pigs manifesting no toxic effects. Intermediate behavior is apparent in the monkey with a whole-body half-life of 8 days after a single i.v. dose of 30.6 μg/kg. In all 3 species, excretion of unmetabolized 2,3,7,8-TCDF is negligible and its metabolism appears to represent a detoxification process. Excretion is predominantly by feces.

Highly chlorinated isomers accumulate in the liver and adipose tissue of humans and persist for many years. 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF are retained by the liver at higher concentrations than other isomers. They have been detected in the liver and the adipose tissue of Yusho and Yu-Cheng patients many years after intoxication. PCDFs and PCDDs have been assayed in tissues of patients 2 years or more after exposure in a building contaminated with these compounds; levels of PCDFs were elevated, especially those isomers found in the building. Many of these were similar to those found in Yusho and Yu-Cheng patients. Surprisingly, high levels of 2,3,7,8-chlorine substituted congeners have been found in adipose tissue and in breast milk of the general population from highly industrialized countries of Europe and North America. Lower levels have been detected in populations from North Vietnam that was considered not contaminated by phenoxy acid herbicides and that had a low level of industrial contamination. Therefore levels of PCDFs in fat biopsies should be considered as a useful biological indicator of levels of exposure for epidemiological studies.

Studies establishing the human metabolism and the kinetics of these compounds are still needed. The kinetics of the more persistent hexa- and hepta-isomers warrant intensive investigations. When the general population is exposed to unknown contamination through the food chain and air, subjects with a high body burden can transmit toxic PCDFs to their progeny through placenta and milk, indicating that such babies are a population at high risk.

7.1.2. Toxicity. Some PCDFs have high acute toxicity. The 2,3,7,8-TCDF, and 1,2,3,7,8- and 2,3,4,7,8-PeCDFs are some of the most acutely toxic isomers. The acute oral LD_{50} of 2,3,7,8-TCDF is 5-10 µg/kg in guinea pigs (2-4 times higher than 2,3,7,8-TCDD), >6000 µg/kg in the mouse and the rat (20 times higher than 2,3,7,8-TCDD), and 1000 µg/kg in the rhesus monkey. The symptoms of toxicity were very similar to those reported for 2,3,7,8-TCDD; for example, thymic atrophy in all species; liver pathology in rabbits, rats and mice; hydropericardium in chicks; alteration of skin and adnexa, liver bile duct and gastrointestinal tract in addition to hyperplastic and metaplastic changes in the meibomian glands of the eyelid and the ceruminous glands of the ear canal in the rhesus monkey. The eyelid and ear canal are the most sensitive indicators of PCDF toxicity in the monkey. There is a lag of several days between administration and the appearance of clinical toxic responses. Two of three monkeys died on a diet containing 5 µg/kg (food) administered over 6 months. The acute LD_{50} values, in µg/kg, for Hartley strain guinea pigs over 30 days are as follows: 2,3,7,8-TCDF (7); 2,3,4,7,8-PeCDF (<10); 2,3,7,8-TBDF (<15); and 2,3,4,6,7,8-HxCDF (120).

It is highly probable that continuous exposure at low levels causes cumulative toxic effects, especially in view of the lesions observed in monkeys at an acute dose, which was <4% of the LD_{50}.

Few studies relate to the subacute toxicity of PCDFs. The most complete data concern the effect of 2,3,7,8-TCDF on mice, chicken, guinea pigs and rhesus monkeys. Common signs for various species seem to be a decrease in body weight and involution of the thymus. In some species (rodents and monkeys) liver morphological lesions were observed such as changes of endoplasmic reticulum, cytoplasmic lipid droplets and mitochondrial alterations. These ultrastructural effects should be considered with particular attention and carefully controlled in planned studies using single isomers, as such alterations have been recently observed in the liver of patients known to be exposed to PCDFs. Ultrastructural alterations of liver mitochondria in liver biopsies, together with the monitoring of the contaminants in fat biopsies, are useful biological markers for exposure to PCDF and related chemicals.

It appears likely that PCDFs contribute substantially to the toxicity of commercial PCBs and polychlorophenols and are responsible for the symptomatology of Yusho and Yu-Cheng diseases.

Dermal toxicity is a common effect in animal species including humans. However, the different species develop different types of skin lesions. After treatment with various mixtures of PCBs, rabbits and rats develop lesions resembling the chloracne observed in Yusho and Yu-Cheng patients.

Information on PCDF effects on the immune system is still limited, although reduction in thymus weight and humoral antibody production has been observed in all the animal species tested. The results with pure PCDF isomers show that the pattern of immunotoxicity for PCDF-active isomers is similar to that of the corresponding PCDD isomers, provided that higher dosages are given to laboratory animals. Data obtained with 2,3,7,8-TCDD in mouse strains eliciting different sensitivities to induction of aryl hydrocarbon-hydroxylase (AHH), suggest that the individual genetic background may play a role in the degree of PCDF-induced immunotoxicity.

A number of PCDF isomers show the capacity to cause a relatively high degree of induction of some liver mixed function oxygenases, such as AHH and ethoxyresorufin-o-deethylase (EROD) both *in vivo* and *in vitro*. AHH induction activity has two structural requirements: first, halogen atoms must occupy at least 3 of the 4 lateral ring positions (2, 3, 7 and 8); second, at least 1 vicinal carbon position must be unsubstituted. Structureactivity studies conducted *in vitro* demonstrated the existence of a broad difference in potency of PCDF induction. The lack of kinetic studies in rodents does not allow precise comparisons to be made between *in vitro* and *in vivo* results. However, recently good correlations were obtained by plotting the inducing capacity of AHH of a single PCDF congener versus the rat body weight loss or thymic atrophy. This approach seems to be particularly useful for testing mixtures of PCDFs with an unknown exact composition.

Biochemical studies have shown that for the manifestation of their inducing effect PCDFs must bind to a cytosolic protein binding site. The occupancy of the receptor seems to be crucial. This event not only causes AHH and EROD induction but (more important) represents the first step triggering the toxic effects displayed by these compounds. Recent results on on the interaction of TCDD with EGF receptor or with receptor protein present in the thymic epithelial cells reinforce this point of view. So far there are no indications that AHH induction could be a cause of toxicity per se, but this fact could be an indication of the potential susceptibility of this organism to more profound toxic signs.

7.1.3. Epidemiology. Information on the toxic effects of PCDF in man comes mostly from Japan and Taiwan where in 1968 and 1979, respectively, many people ate rice oil that was later found to be contaminated with PCBs, PCDFs and PCQ. More than 4000 people in the two episodes were intoxicated.

Some general symptoms included: retarded growth, abnormal lipid metabolism, liver disturbances, acneform eruption, skin pigmentation and cutaneous lesions. The clinical profile of this syndrome has been well defined but long-term toxicity, carcinogenic risk and mortality rate consequent to PCDF exposure have not yet been assessed.

7.2. INTRODUCTION

The toxicity of PCDFs in laboratory animals has received attention as a consequence of their identification in widely used industrial chemicals, such as the PCBs and polychlorophenols. Evidence is accumulating that many of the toxic effects of various commercial chemicals may actually result from traces of PCDFs. The critical observation leading to the hypothesis that a trace contaminant might be the major toxic factor was made by Vos and Koeman (1970) who found significant differences in toxicity between three commercial PCB preparations. Later, Vos et al. (1970) showed that most acutely toxic preparation contained TCDFs and PeCDFs. Out of 135 possible isomers of PCDFs only 10-12 are expected to have significant acute toxicity. The most acutely toxic isomers appear to be 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF (McKinney et al., 1976; Poland et al., 1976; Moore et al., 1979). Dibenzofuran (DF) and OCDF have low acute toxicity (Goldstein et al., 1978b). Toxicological studies of PCDFs reveal marked similarities to the effects of PCDDs (Moore et al., 1979; WHO, 1978b). The estimated difference in toxicity between 2,3,7,8-TCDD and 2,3,7,8-TCDF is at least 10-fold (Kimbrough, 1974; Kimbrough et al., 1978; Moore et al., 1979), but it is consistently dependent on the animal species considered and on the specific parameter investigated. The earlier studies were essentially devoted to identifying the acute toxic effects. The series of incidents involving a large number of people from the general population or from exposed workers in which PCDFs have been implicated as the major class of compounds responsible for the toxic effects have prompted researchers to design more extensive studies on the kinetics, subchronic toxicity and biochemical mechanisms. Therefore, studies on long-term effects of these chemicals are warranted.

7.3. TOXICOKINETICS

7.3.1. Introduction. The disposition of the PCDFs in animals depends on the physicochemical properties of the single isomers. Their relative lipid solubility, which increases with increasing halogenation, promotes their passive absorption from the gastrointestinal tract but impedes their excretion directly in the urine and the bile. In addition, since PCDFs are lipophilic, animals with high adipose weight/body weight ratios tend to retain them. Thus, metabolism is the rate-limiting factor in the fate of PCDFs in animals. A particular aspect that must be considered in assessing the bioavailability is the influence of environmental matrices, route of administration and vehicles when PCDFs come from environmental pollution or from contamination of chemicals (Kaminsky et al., 1985).

Metabolism is also dependent on molecular characteristics, since the less highly halogenated isomers and those with unsubstituted vicinal carbon atoms are more readily metabolized. In all animal species studied, the metabolites, and to some extent the parent compound, are excreted preferentially in the feces, through the bile and to a lesser extent in the urine.

Data on distribution, metabolism and excretion of 2,3,7,8-TCDF are available for several different animal species. Recently, a physiological pharmacokinetic model was developed for 2,3,7,8-TCDF (King et al., 1983).

7.3.2. Absorption. PCDFs are lipid soluble compounds and are absorbed from the gastrointestinal tract by passive diffusion across cell membranes.

In rats (Birnbaum et al., 1980, Decad et al., 1982), mice (Decad et al., 1981a; 1982) and guinea pigs (Decad et al., 1981b; 1982) 2,3,7,8-TCDF appears to be absorbed to a very high degree. In guinea pigs, absorption of TCDF was estimated at >95% in the gut after an oral exposure.

As for other halogenated compounds (Damstra et al., 1982) the absorption tends to decrease with increasing halogenation above a certain point. For the 1,2,4,6,8,9-HxCDF the degree of absorption seems to be lower than for TCDF (Birnbaum, 1985). That highly halogenated PCDFs could be less easily absorbed has been suggested but not investigated because of the lack of appreciable amounts of the various congeners needed for these studies.

Absorption can also be strongly influenced by the vehicle. In the guinea pig acute toxicity of 2,3,7,8-TCDD (LD_{50}) is approximately one-eighth of its LD_{50} in corn oil (Silkworth et al., 1982) and 3-5 times higher levels of TCDD are needed to cause toxic effects when they are administered mixed with dirt (McConnell et al., 1984). It has been suggested that similar differences may also apply to PCDF (Silkworth et al., 1982). However, direct evidence is lacking for PCDF. Berg (1985b, 1986) report that the bioavailability of PCDF absorbed on fly ash of a municipal incinerator and administered with the diet was very scant in different animal species.

7.3.3. Distribution, Metabolism and Excretion. Disposition has been studied in various animal species. However, a critical comparative analysis of the results is difficult as PCDF mixtures of variable (and in some cases unknown) composition have been used and the experimental conditions, doses, routes of administration, duration and schedules of data recording differ. Only a few trials used single purified PCDF isomers. However, from all these data, which are summarized in the following sections, it appears that liver and adipose tissue are the preferential tissues for localization, that elimination is by metabolism, and that metabolites, once formed, are excreted without delay.

7.3.3.1. RAT -- Kuroki et al. (1980) used a PCDF mixture (14% 1,2,7,8-TCDF; 35% 2.3.7.8-TCDF; 1% 1,2,4,7,8-PeCDF; 49% 1,2,3,7,8-PeCDF; 1% 2,3,4,7,8-PeCDF; and 1% unspecified HeCDF) to investigate the persistence of PCDF in the liver of rats and monkeys. Four male Wistar rats (~100 g and 4 weeks of age) were given a single i.p. dose of PCDF 10 mg/kg bw. The rats were killed after 5 days and their livers were removed for GC/MS assay of PCDF. The data are presented in Table 7-1. The 1,2,7,8-TCDF and 1,2,4,7,8-PeCDF were not detected, and the 2,3,7,8-TCDF was present at 0.6-2/5% of the total dose. The amounts of 2,3,4,7,8-PeCDF and HeCDFs retained in the liver were 76-82% and 100% of the total dose, respectively. Thus, 2,3,7,8-TCDF was eliminated from the liver in these rats with a residence half-time of ~1 day.

Birnbaum et al. (1980) and Decad et al., 1982 investigated the absorption, distribution and excretion of radiolabeled 2,3,7,8-TCDF in 200-500 g male Fisher 344 rats. The compound was administered at oral doses of 30.6 (0.1 μmol/kg) and 306 μmol/kg (1.0 μg/kg bw), and at an i.v. dose of 30.6 μg/kg bw. About 90% of both oral doses was absorbed. Table 7-2 shows that the adrenals, liver, lungs and kidneys contained the highest level of radioactivity 15 minutes after the i.v. injection (0.1 μmol/kg bw). Subsequent redistribution to adipose tissue followed, the concentration was maximum there 7 hours after administration. The pattern of distribution of radiolabel in liver fat muscle, skin and blood, and the amounts excreted in urine and feces 3 days after administration were essentially independent of the dose and route of administration (Table 7-3). No evi-

TABLE 7-1

Percentages of the Total Intake of Original PCDF Isomers Found in the Livers of Monkeys and Rats[a,b]

	CDF					
Mixture Administered	1,2,7,8- (14%)	2,3,7,8- (35%)	1,2,4,7,8- (1%)	1,2,3,7,8- (49%)	2,3,4,7,8- (1%)	Hexa- (1%)
Male Wistar Rat (100 g)						
1	ND	1	ND	18	81	109
2	ND	1.5	ND	17	80	104
3	ND	2.5	ND	21	82	108
4	ND	0.6	ND	14	76	107
Mean ± S.D.	ND	1.4±0.8	ND	18±3	80±3	107±2
Rhesus Monkey						
1	ND	0.02	0.4	0.2	5.2	0.8
2	ND	0.09	0.3	0.2	1.7	0.1
3	ND	-	-	0.2	0.8	ND
Mean ± S.D.	ND	0.04±0.05	0.2±0.2	0.2	2.6±2.3	0.3±0.4

[a] Source: Kuroki et al., 1980

[b] Three monkeys were fed Kanechlor 400 (0.25; 0.5 mg/kg bw) and PCDF (1.25; 2.5 µg/kg bw) for up to 32 days. Rats were injected i.p. with 10 mg PCDF/kg bw and killed after 5 days. The total ingested Kanechlor 400 was 86, 88 and 30 mg in monkeys designated 1, 2 and 3, respectively; the total ingested PCDF was 430, 440 and 151 µg, respectively. Levels of Kanechlor in the liver were 1.7, 0.8 and 0.04 ppm, and of PCDF 5.4, 4.2 and 1.1 ppb, respectively. Levels of Kanechlor in fat were 8.6, 25 and 1.2 ppm, and of PCDF 0.9, 26 and 0.2 ppb, respectively.

ND = Not detectable

TABLE 7-2

Concentration of ^{14}C-2,3,7,8-TCDF in Tissues of Male Fisher 344 Rats[a,b]

Tissue	% Total Label (g/tissue basis)		
	15 Minutes	180 Minutes	1 Day
Blood	0.12±0.04	0.04±0.01	0.03±0.01
Liver	4.4 ±0.2	5.1 ±0.4	2.2 ±0.4
Fat	0.20±0.03	0.44±0.07	0.64±0.11
Muscle	0.25±0.01	0.06±0.00	0.30±0.02
Skin	0.17±0.02	0.20±0.01	0.07±0.01
Kidneys	0.67±0.04	0.17±0.03	0.08±0.01
Adrenals	7.4 ±6.9	4.7 ±1.3	0.34±0.14
Thyrus	0.52±0.12	0.54±0.13	0.07±0.03
Spleen	0.37±0.07	0.08±0.02	0.02±0.00
Testes	0.09±0.01	0.09±0.01	0.06±0.02
Brain	0.25±0.01	0.15±0.03	0.02±0.00
Lungs	1.08±0.08	0.24±0.02	0.07±0.02
Heart	0.66±0.03	0.11±0.00	0.02±0.02

[a] Source: Birnbaum et al., 1980

[b] Dosed with 0.1 μmol/kg bw i.v.

dence of enterohepatic circulation was found Examination of tissue extracts showed that nearly all radiolabel in blood and tissues, at least up to 1 day postadministration, was present as unchanged 2,3,7,8-TCDF. Thus, the half-time of radiolabel loss from selected tissues showing a biphasic rate (Table 7-4) represent half-times of loss of 2,3,7,8-TCDF. The loss from skin was biphasic, the early component having a half-time comparable to other tissues (see Table 7-4), followed by the slow component with a half-time of ~11 days, which accounted for <1% of the total dose.

In contrast with blood and tissues, >99% of the radiolabel excreted in the feces and urine was associated with metabolites of 2,3,7,8-TCDF. Since the metabolites appeared to be excreted as soon as they were formed, the half-times of radiolabel in the urine (1.3 days) and feces (1.8 days) represent the half-time of elimination (by metabolism) of 2,3,7,8-TCDF. These values agree with the half-times in blood and liver (see Table 7-4). The feces was the primary route of excretion, accounting for ~85% of the total dose, while <6% was excreted in the urine. The ratio of kidney to bile clearance was 0.03.

Yoshimura et al. (1984) studied the tissue and subcellular distribution of 2,3,4,7,8-PeCDF given orally to the rat. More than 60% of the dose was accumulated in the liver after 5 days and persisted over a period of 3 weeks. The small percentage of radioactivity distributed in all other tissues was eliminated quickly. The subcellular liver distribution of PeCDF was parallel to that of cytochrome P450. Excretion of the unchanged compound in the feces amounted to ~32% of the dose during the first 24 hours with progressively smaller amounts during the following 3 weeks. No excretion was recorded in urine up to day 21. The fecal excretion of PeCDF can be increased by treatment with activated charcoal beads (Yoshimura et al.,

TABLE 7-3

Distribution of Radioactivity 3 Days After Administration of ^{14}C-2,3,7,8-TCDF to 200-250 g Male Fisher 344 Rats*

Tissue	Percentage Total Dose		
	Oral		i.v.
	1.0 μmol/kg	0.1 μmol/kg	0.1 μmol/kg
Liver	3.9±2.2	5.0±0.4	5.9±0.3
Fat	9.8±3.6	4.6±0.8	12 ±2
Skin	1.1±0.6	1.1±0.2	1.2±0.3
Total excreted In feces	68 ±6	70 ±4	63 ±10
In urine	1.5±0.3	1.76±0.01	2.0±0.4

*Source: Birnbaum et al., 1980

TABLE 7-4

Components for the Elimination of Radioactivity from Tissues of Fisher 344 Rats[a]

Tissue	Component	Pool Size (% total dose)	Decay Rate (day^{-1})	Mean Half-Time (days)
Blood	1	1.3±0.4	32 ±22	0.02
	2	0.9±0.2	0.6 ±0.3	1.1
Liver	1	29 ±6	7.0 ±2.7	0.10
	2	31 ±3	0.55±0.08	1.3
Fat	1	18 ±1	0.19±0.04	3.8
Muscle	1	25 ±2	29 ±6	0.024
	2	6.7±1.5	0.96±0.50	0.7
Skin	1	6.8±0.5	1.6 ±0.3	0.45
	2	1.2±0.4	0.06±0.41	11[b]

[a] Source: Birnbaum et al., 1980

[b] Complex redistribution occurs making the calculation of an S.D. tenuous.

1986). Such treatment in rats for 3 weeks stimulated excretion ~3-fold and significantly suppressed PeCDF toxicity (thymus atrophy and liver hypertrophy). PeCDF levels in blood, adipose tissue and, to a lesser extent, in liver, tended to decline.

Recently, Brewster and Birnbaum (1986) found that the disposition of 2,3,4,7,8-PeCDF in the rat was similar in animals given either an oral or i.v. dose; they confirmed the greater accumulation of the administered chemical in liver. Fat, skin and muscles were found to be minor depots. Excretion by feces was <1% of the administered dose per day and <0.5 in the urine within 3 days. No PCDF was detected in expired air.

Data from Birnbaum (1985) indicate that 1,2,3,6,7,9-HxCDF is not easily metabolized by rats, compared with the TCDFs and PeCDFs and is excreted in feces less than TCDF (ratio feces/urine 13 for HxCDF and 31.4 for TCDF). The half-life for elimination has been calculated at 13 days. These results confirm the data obtained by Kuroki et al. (1980) who showed that rats eliminate 2,3,7,8-TCDF and retain PeCDFs and HxCDFs.

The few data available indicate that in the rat PCDFs concentrate in liver more than in fat with a tendency for higher levels of halogenation to result in higher liver concentrations (liver/adipose % total dose 1.30 for 2,3,7,8-TCDF, 1.8 for 1,3,4,5,7,9-HxCDF) (Birnbaum, 1985).

7.3.3.2. MOUSE -- Morita and Oishi (1977) gave male ICR mice a single i.p. dose of a mixture of 0.5 g PCDF in corn oil. The mixture contained unspecified 2-TCDFs, 4-PeCDFs and 4-HxCDFs. The less chlorinated PCDFs were eliminated from tissues faster than the highly chlorinated ones. The half-times in tissues were a week or less.

Decad et al. (1982) studied the distribution and excretion of 2,3,7,8-TCDF in two strains of mice given 30.6 μg i.v. radiolabeled 2,3,7,8-

TCDF/kg bw. Higher concentrations of radiolabel were observed in the liver than in any of the other 12 tissues sampled up to 10 days postadministration. DBA/2J mice had ~1.6 times as much adipose tissues as C57Bl/6J mice, and the nature of adipose tissue radiolabel kinetics differed considerably in the two strains, with the concentration of radiolabel peaking later and at a higher level in the adipose tissue of DBA mice. Nearly all the tissue radiolabel was unmetabolized 2,3,7,8-TCDF, while essentially all of the radiolabel in urine and ~80% of the radiolabel in feces, after the first postadministration day, represented metabolites. The whole-body residence half-time, calculated from the rates of excretion of radiolabel in the urine and feces, was ~2 days in C57Bl/6J mice and ~4 days in DBA/2J mice (Table 7-5).

Nagayama et al. (1980) studied the transfer of PCDFs to the fetuses and offspring of ddN mice (25 g bw) fed a diet containing 0.6 ppm of a mixture of PCDF (48% TCDFs, 49% PeCDFs and 3% HxCDFs) for 18 days after mating and 14 days after delivery. Accumulation of PCDF in the fetuses across the placenta was only 0.003% of the total amount ingested by the dam. There was much greater transfer of PCDF to suckling offspring (1.2% of the total amount ingested by the dam). PCDF accumulated more in the dams' livers (5.1% of the total amount ingested) than in other tissues. Data from Weber and Birnbaum (1985) confirmed that <0.1% of the total dose appeared in the fetuses 1 day after acute exposure on day 11 of gestation.

7.3.3.3. MONKEY -- The PCDF mixture reported in Section 7.3.3.1. was given orally, in combination with a commercial PCB mixture, to 1 female and 2 male monkeys daily for 26-32 days, after which the monkeys were killed and adipose tissue and liver analyzed for their content of PCDF isomers (Kuroki

TABLE 7-5

Excretion of 2,3,7,8-TCDF-Derived Radioactivity by Three Mice*

Mouse Strain	Elimination Route	Mean ± S.D. Cumulative Excretion of Dose by Day 10 (%)	Mean Half-Time (days)
C57Bl/6J	Urine	12.6± 0.1	2.8
	Feces	81.9±13.0	1.8
	Urine + feces	97.0± 9.2	2.0
DBA/2J	Urine	19.9± 4.6	4.9
	Feces	55.8± 4.8	5.4
	Urine + feces	75.7± 5.8	4.0

*Source: Decad et al., 1982

et al., 1980). As in the rat, PeCDFs and HxCDFs were retained at higher concentrations than TCDFs (see Table 7-1).

In the only kinetic study using a single isomer (Birnbaum et al., 1981), three male rhesus monkeys were given 30.6 μg 2,3,7,8-TCDF/kg bw i.v. Loss of radiolabel from blood was monitored only for 18 minutes in order to avoid an extended period of sedation (Ketaset 50 mg i.m.) and to minimize stress. The monkeys were killed 21 days after treatment, and the pattern of radiolabel distribution in the tissues was determined (Table 7-6). Less than 10% of the injected dose remained in the body at this time. As with the rat, all radiolabel extractable from liver, fat, skin and muscle was unmetabolized 2,3,7,8-TDCF, but about one-third of the small amount of extractable radiolabel in blood did not co-chromatograph with the parent compound and may have been associated with metabolites. Only metabolized 2,3,7,8-TCDF was extracted from the urine, and nearly all radiolabel extracted from the feces represented metabolites. Thus, the half-times of radiolabel in urine (6.2 days) and in feces (10.3 days) represent estimates of the whole-body half-time. Eight percent of the dose was excreted in the urine and 43% in the feces.

King et al. (1983), using the data for the monkey (Birnbaum et al., 1981), rats (Birnbaum et al., 1980), and mice (Decad et al., 1982), compared the tissue to blood distribution ratios (Section 7.3.3.5.).

7.3.3.4. GUINEA PIG -- The extremely high toxicity of the PCDFs in guinea pigs (Moore et al., 1979) makes it difficult to obtain reliable information concerning their disposition. Decad et al. (1981b) reported that the distribution of 2,3,7,8-TCDF radiolabel in guinea pigs during the first 24 hours after either oral or i.v. administration of 6 μg/kg bw (near the LD_{50}) was similar to that in other species, with liver, fat,

TABLE 7-6

Distribution of 2,3,7,8-TCDF-Derived Radioactivity in Three Rhesus Monkeys 21 Days After Treatment[a]

Tissue	Percentage Total Dose[b]	pmol TCDF/g Tissue[c]
Blood	0.37±0.02	4.8± 0.3
Liver	1.0 ±0.8	47 ±30
Skin	2.4 ±1.6	12 ± 6
Fat	3.7 ±2.8	47 ± 0.2

[a] Source: Birnbaum et al., 1981

[b] i.v., 0.1 μmol/kg bw

[c] Mean ± standard deviation

muscle and skin containing much of the radiolabel, but the ratio of liver to adipose tissue was lower than in the rat (Table 7-7). During the second 24-hour period, however, redistribution to the liver began. Subsequently, the amount of radiolabel in the liver rose to over 50% of the total administered amount by the ninth day postadministration. This redistribution and accumulation was at least partly the consequence of mobilization of body fat, a toxic effect of 2,3,7,8-TCDF. In this study a minimum half-life of 20 days has been indicated, but in view of this fat loss with release of radiolabel, no reliable half-life can be calculated from these data. In fact in a more recent study by Ioannou et al. (1983) the half-life calculated on data from animals showing little or no significant effects of TCDF intoxication was ~40 days. In a multiple oral treatment the concentration in adipose tissue was proportional to the dose. With increasing time after dosage, the distribution starts to shift from adipose tissue to liver.

The amounts of radiolabel excreted in the urine and feces of these guinea pigs were comparable, only ~6.6% being excreted by each route by the ninth day after i.v. injection. While radiolabel was excreted in the urine as metabolites, >90% of the radiolabel excreted in the feces was unmetabolized 2,3,7,8-TCDF. Thus, although urinary excretion by these guinea pigs appeared comparable with that in other species at exposures that do not cause toxicity, fecal excretion was markedly lower compared with the other species and was restricted largely to parent 2,3,7,8-TCDF.

Attention has been paid in recent years to the bioavailability of PCDF from different matrices. Berg et al. (1985b) studied its availability from fly ash of a municipal incinerator in the rat, hamster and guinea pig. Fly ash was mixed with the standard laboratory diet and animals were allowed to eat ad libitum for 3 months. Contamination of the PCDF isomers was in the

TABLE 7-7

Distribution of 2,3,7,8-TCDF-Derived Radioactivity During the First 24 hours in Liver, Fat and Skin of Guinea Pigs[a,b]

Time After i.v. Injection (hours)	Percentage of Total Dose[c]		
	Liver	Fat	Skin
1	43.1±1.4	27.4± 7.6	14.6±0.9
3	23.6±3.8	31.4± 0.7	22.5±0.1
7	17.8±0.8	68.5±11.3	20.9±1.8
24	15.4±3.7	55.2±19.8	19.4±3.3

[a] Source: Decad et al., 1981b

[b] Hartley guinea pigs received a single i.v. dose of (^{14}C)-2,3,7,8-TCDF 0.02 μmol/kg. Three animals were killed at each time 1, 3, 7 and 24 hours, respectively.

[c] Mean ± S.D.

range of 0.5-3.6 μg/g food. Rats and hamsters retained mainly the isomers with 2,3,7,8-substitution patterns whereas guinea pigs retained also a number of other substituted isomers probably because of less effective metabolism. Usually recovery in the liver was below 10% for all three species. In a subsequent paper Berg et al. (1986) gave rats and hamsters oral treatments with fly ash at higher concentrations (15% in food compared with the earlier concentrations of 2.9%); they found higher liver retention, from 20-60% for penta and hexa congeners. In rats, retention was highest for 1,2,3,7,8-PeCDF (41%) and very low for 2,3,7,8-TCDF (1.1-2.8%); in hamsters the highest retention was for 2,3,4,7,8-PeCDF (71%) and ~38% for 2,3,7,8-TCDF. The authors suggested that the hamster probably metabolizes TCDF less efficiently than the rat. As a whole, these results indicate that the bioavailability for PCDF, when absorbed on fly ash, is very low.

7.3.3.5. PHYSIOLOGICAL DISPOSITION MODEL -- When uptake, accumulation and disposition parameters are known, pharmacokinetic models can be useful to predict the overall distribution and to describe the time course of tissue concentrations and excretion of metabolites. These models can provide a means of estimating the metabolic and excretory clearance, difficult in a complex _in vivo_ system.

The disposition data for 2,3,7,8-TCDF in the mouse (two strains) (Decad et al., 1982), rat (Birnbaum et al., 1980) and monkey (Birnbaum et al., 1981) were compared by King et al. (1983) with simulated data generated by a physiological pharmacokinetic model. In developing the model, King et al. (1983) used tissue-to-blood concentration ratios from the studies previously discussed in Section 7.3.3.3. to estimate partition coefficients. Metabolic clearances were calculated from the data sets as cumulative total radiolabel excreted (urine and feces) divided by the total area under the curve of concentration in hepatic venous blood. Values of these parameters are given in Table 7-8.

Concentrations predicted by the physiological model generally compared well with measured concentrations in all three species. Metabolic clearance displayed the dependence on body weight to the power of 0.7 that is characteristic of many physiological functions. Extrapolation to man on this basis yields a whole-body half-life estimate of 5-12 days for 2,3,7,8-TCDF. This indication that TCDF would not be expected to persist for long periods in man does not disagree with the values determined in tissues of patients exposed to PCDF. In fact 2,3,7,8-TCDF was less persistent than the higher chlorinated hexa and hepta congeners. However, the whole body half-life seems to be greatly underestimated. In general the possibility of extrapolating these pharmacokinetic data is limited by the fact that metabolism varies between species and particularly as far as PCDF metabolism is concerned, the differences and similarities in the metabolic fate in humans and laboratory animal species are not yet satisfactorily distinguished.

7.3.3.6. MAN -- Pharmacokinetics of PCDFs in man (including absorption, metabolism, excretion and half-lives) are unknown. However, PCDFs have been detected in tissues of subjects exposed in different ways to these compounds. Measurements of tissue levels of the various isomers over time provide a gross estimate of the extent of exposure and can help to elucidate the structural rules governing the metabolism and elimination of these classes of compounds. As methods are now available to detect dibenzofuran isomers in human tissues in the ppt range (Albro et al., 1985), measurement of PCDFs in fat biopsies can be considered a biological marker of exposure. PCDFs have been detected in man after different types of exposure to recognized sources of contamination and, very recently, also in the general popu-

TABLE 7-8

2,3,7,8-TCDF Pharmacokinetic Parameters for Physiological Model[a]

	Mouse			
	C57	DBA	Rat	Monkey
Distribution ratio[b]				
Liver	100	100	100	30
Fat	25	40	35	30
Skin	8	12	4	7
Muscle	2	4	2	2
Clearances				
Metabolism[b] k_m(mℓ/min)	0.07	0.06	1.0	2.25
Metabolites excretion ratio k_k/k_e	0.14	0.27	0.03	0.19

[a] Source: King et al., 1983

[b] For parent TCDF

lation that is evidently exposed to unknown contaminations. Depending on the higher detection limit of the method used in the first reports, in many cases not indicated, the concentrations in these studies are not comparable, particularly the levels in control subjects, but the earlier results are reported here when they are significant in the context of the scientific merit of recent observations.

PCDFs have been found in tissues of Yusho and Yu-Cheng patients intoxicated in Southwest Japan (1968) and in Taiwan (1979), respectively by consumption of a commercial rice oil contaminated with high concentrations of PCBs, PCDFs and PCQs. Although the PCDF concentrations in the Taiwan rice oil were lower than in the Japanese oil, (Table 7-9) it has been roughly estimated that the average intake during the whole intoxication period was similar: 973, 3.8 and 586 mg in Taiwan (Chen et al., 1985a) and 633, 3.3 and 596 mg in Japanese patients (Hayabuchi et al., 1979) for PCBs, PCDFs and PCQs, respectively. An example of the tissue PCDF distribution with the relative concentrations of the various isomers detected was obtained by the analysis of samples from a Taiwanese patient who died ~2 years after the onset of poisoning (Chen and Hites, 1983). The PCDF congeners retained in all the tissues were essentially the same, with higher levels in liver. The relative amounts of the individual PCDF congeners differed in liver and other tissues (Table 7-10). The major congeners detected were 1,2,4,7,8- and 2,3,4,7,8-PeCDFs; 1,2,3,4,7,8-HxCDF. Minor amounts of 2,3,7,8-TCDF and 1,2,3,4,6,7,8-HpCDF were found. All the PCDFs retained have at least 3 chlorine atoms at 2,3,7,8 positions and no vicinal hydrogens in the dibenzofuran ring. As stated in Section 7.4., these bioaccumulative PCDFs also strongly induce benzo(a)pyrene hydroxylase and DT-diaphorase and cause severe atrophy of the thymus and significant hypertrophy of the liver in

TABLE 7-9

Concentrations of PCBs, PCDFs and PCQs in the Rice Oils from Japan and Taiwan[a]

	Concentration (ppm)		
		Taiwan Rice Oil	
	Japan Rice Oil	A	B
PCBs	900	60	100
PCDFs			
Unknown tetra-	0.65	0.04	0.05
2,3,7,8-tetra-	0.20	trace	trace
2,3,4,7,8-penta-	0.70	0.02	0.02
2,3,4,6,7-penta-	0.35	0.01	0.02
hexa-	0.12	0.01	0.01
TOTAL	2.02	0.08	0.10
PCQs	800[b]	90[b]	180[b]

[a] Source: Masuda et al., 1982

[b] Approximate concentration

A and B = two different oil samples

Trace = 0.001-0.005 ppm

rats (Yoshihara et al., 1981; Nagayama et al., 1983); and these isomers can be considered the most toxic compounds, at least in animals.

No congeners with adjacent unsubstituted carbon atoms have been detected in tissues of the Yu-Cheng patient (see Table 7-10). By contrast these unhalogenated vicinal-C-atom congeners were present in the rice-bran oil that the patient had ingested (Table 7-11). A similar pattern of isomers had been detected by Rappe et al. (1979b) in a Japanese Yusho patient who died 18 months after the exposure. Thus, it has been suggested that congeners with at least two vicinal hydrogens in the ring are metabolized or excreted during the interval. Some other autopsy results (Kuroki and Masuda, 1978) are provided in Table 7-12. Preferential accumulation in the liver, followed by adipose tissue sequestration for the isomers with Cl substitution in the 2,3,7,8-positions, was also observed (Rappe et al., 1983b) in tissues of a Taiwan Yusho baby (Table 7-13).

A correlation between the severity of clinical symptomatology in Yusho patients and the estimated contaminated oil ingestion (PCBs + PCDFs + PCQs) was reported promptly (Kuratsune et al., 1972; Nagayama et al., 1976) (Table 7-14). However there is much evidence to support the hypothesis that PCDFs and not PCBs are responsible for the disease. Analysis of the concentrations of PCDF and PCB in the liver and adipose tissue of Yusho patients and of control subjects killed in traffic accidents revealed comparable PCB concentrations in tissues of the two groups, but PCDF (in the range of ppb) only in the organs of Yusho patients (Masuda and Kuroki, 1982). Other evidence of the importance of PCDF and PCB in determining Yusho and Yu-Cheng syndrome has been obtained more recently. Kashimoto et al. (1985) compared the blood levels of Yusho (11 years after the outbreak) and Yu-Cheng patients with that of occupationally PCB exposed workers (19 years after

TABLE 7-10

Concentrations of PCDF Congeners in the Tissues of the Deceased Patient with Yu-Cheng in Taiwan*

Tissue	Level of PCDF Congener (ppb)		
	1,2,4,7,8-	2,3,4,7,8-	1,2,3,4,7,8-
Liver	3.4	6.3	25.4
Intestinal fat	0.9	4.0	7.8
Bronchus	0.4	1.8	3.2
Large intestine	0.3	1.2	2.3
Heart	0.2	0.8	1.4
Stomach	0.05	0.23	0.40
Small intestine	0.05	0.21	0.34
Kidney	0.04	0.18	0.32
Lung	0.01	0.06	0.12
Brain	0.01	0.06	0.15
Spleen	0.01	0.08	0.10

*Source: Chen and Hites, 1983

TABLE 7-11

Structural Assignments of PCDF Congeners in the Toxic Rice-Bran Oil Ingested by the Deceased Yu-Cheng Patient*

Structure
2,3,6,8-TCDF
2,3,4,8-TCDF (major)
2,3,7,8-TCDF (minor)
1,2,4,7,8-PeCDF
1,2,3,4,8-PeCDF
1,2,6,7,8-PeCDF
2,3,4,7,8-PeCDF
2,3,4,6,7-PeCDF
1,2,3,4,7,8-HxCDF
1,2,3,4,6,7-HxCDF
1,2,3,4,6,7,8-HpCDF

*Source: Chen and Hites, 1983

TABLE 7-12

PCB and PCDF Concentrations in Liver and Adipose Tissue of Deceased Yusho Patients[a] (Exposure was in May-June 1968)

Case	Time of Death	Tissue	PCB Concentration (ppm)	PCDF Concentration (ppb)[b]					
				A	B	C	D	E	Total
1	July 1969	liver	0.14	0.7	0.3	7.1	6.9	2.6	18.0
2	July 1969	liver	0.20	0.08	0.02	0.4	1.2	0.3	2.0
		adipose	2.8	0.6	0.3	1.0	5.7	1.7	9.3
3	May 1972	liver	0.03	0.03	0.005-0.01	0.09	0.3	0.03	0.45
		adipose	4.3	0.08	<0.005	0.2	0.8	0.2	1.3
4	April 1975	adipose	0.2	0.4	<0.005	0.8	0.1	0.5	1.8
5	March 1977	liver	0.006	<0.005	<0.005	0.02	0.1	0.04	0.16
		adipose	1.2	<0.005	<0.005	0.2	0.5	<0.005	0.7

[a] Source: Kuroki and Masuda, 1978

[b] A = 2,3,6,8-TCDF
B = 2,3,7,8-TCDF
C = 1,2,4,7,8-PeCDF
D = 2,3,4,7,8-PeCDF
E = 1,2,3,4,7,8-HxCDF + 1,2,3,6,7,8-HxCDF

TABLE 7-13

Levels of PCDF (pg/g) and PCB (ng/g) in Tissue Samples of a Baby from a Woman from Taiwan Suffering from Yusho Disease*

Isomer	Adipose Tissue	Liver	Muscle	Omentum	Diaphragm
2,3,7,8-TCDF	17	60	ND	ND	ND
1,2,4,7,8-PeCDF	14	42	ND	ND	ND
1,2,3,7,8-PeCDF	44	194	ND	ND	ND
2,3,4,7,8-PeCDF	68	91	ND	ND	ND
1,2,3,4,7,8-HxCDF	88	193	ND	ND	ND
PCDF	231	580	ND	ND	ND
PCB	316	27	38	64	46

*Source: Rappe et al., 1983b

ND = Not detected

TABLE 7-14

Relationship Between the Amount of Rice Oil Used by Yusho Patients, Amounts of PCBs and PCDFs Ingested, and Clinical Severity[a]

Rice Oil Consumed (mℓ)	Consumed[b]		Number of People[c]			
	PCBs (g)	PCDFs (mg)	Unaffected	Light Cases	Severe Cases	Total
<720	<0.7	<3.6	10 (12.0)	39 (49.0)	31 (39.0)	80
720-1440	0.7-1.4	3.6-7.2	0	14 (31.0)	31 (69.0)	45
>1440	>1.4	>7.9	0	3 (14.0)	18 (86.0)	21

[a] Source: Kuratsune et al., 1972; Nagayama et al., 1976

[b] Calculated on the basis of the concentrations of PCBs (1000 ppm), PCDFs (5 ppm) found in the contaminated rice oil by Nagayama et al. (1976)

[c] Percentage of cases is given in parentheses.

termination) and unexposed people. In spite of high levels of PCBs in all the samples, detectable amounts of PCDFs were only found in blood of Yu-Cheng patients. In 113 Yu-Cheng patients there was a clear correlation between the blood PCDF concentration and the severity of dermatological symptoms. PCQs were present in blood of all the Yu-Cheng patients 6 months after exposure and in 54 of the 56 living Yusho patients 11 years after the outbreak. So the presence of PCQs in blood can be considered a good marker of past ingestion of contaminated oil.

In the blood of Yu-Cheng patients there was a distinctive PCB pattern, very different from the original pattern (Masuda et al., 1985) and richer in the more chlorinated isomers (for example, 2,3,4,5,3,4-hexa-CBs a PCB with high bioaccumulative properties). This distinctive chromatogram has now been adopted as one of the criteria for identification of Yu-Cheng disease. An example of the levels of three halogenated congeners in tissues of different categories subjects is given in Table 7-15.

Preliminary data in the three Taiwan Yusho patients indicated that in the first year after exposure blood concentrations of penta- and hexa-CDF dropped 20 and 15%, respectively. Thus, the half-time of highly-chlorinated PCDF in man appears to exceed 1 year (Rappe et al., 1983c). Rappe et al. (1979b) actually detected reliable levels of PCDF in blood of Yusho patients 10 years after the intoxication. Of particular interest is the detection of high concentrations (100-500 ppt) of 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF in the placenta of Yusho women 5 years after exposure. The implications of these results in relation to the increased microsomal enzyme activity detected in the placenta of these patients is discussed under Section 7.4.5.5.2. (Wong et al., 1985).

TABLE 7-15

Concentrations of PCBs, PCDFs and PCQs in the Tissues of Yusho Patients, Unexposed Individuals and in the Milk Fat of a Worker Occupationally Exposed to PCBs[a]

Case	Age	Sex	Time After Exposure (years)	Tissue (or fluid)	Concentration (ppb)		
					PCBs	PCDFs	PCQs
Yusho patients							
1	25	M	1	Adipose tissue	5090	5.3	2400
				Liver	226	70.1	218
2	46	F	4	Adipose tissue	6091	9.6	1444
				Liver	68	15.5	144
3	72	M	7	Intestine	3472	2.5	1770
				Liver	114	6.2	51.7
4	59	M	9	Intestine	3630	2.2	416
				Liver	64	6.1	6.3
5	59	M	9	Intestine	1273	0.2	24.5
				Liver	18	0.02	0.97
Unexposed individuals							
Avg.	53	M,F	--	Adipose tissue	803[b]	0.019[b]	1.53[b]
				Liver	33[b]	0.006[b]	0.41[b]
PCB worker							
	37	F	11	Milk fat	6241	0.02	0.2

[a] Source: Miyata et al., 1985

[b] Average concentration from 14 persons

Rappe and Buser (1981) reported measurable levels of PCDF in the blood of workers exposed to 2,3,4,6-tetra-chlorophenol and in workers exposed to pentachlorophenol or pentachlorophenol laurate. Tables 7-16 and 7-17, respectively, report the levels of chlorophenols found in urine samples and of PCDF and PCDD in whole blood. The pattern of PCDD and PCDF in the blood differed in workers exposed to 2,3,4,6-tetrachlorophenol or pentachlorophenol. This parallels the difference in the concentrations of contaminants in the two products: more PCDFs were present in 2,3,4,6-tetrachlorophenol than in pentachlorophenol.

Recently, Rappe et al. (1983b) analyzed samples of liver and kidney tissue of a worker who had been employed in the production of phenoxy acid herbicides. He died of a pancreatic tumor 3-4 years after the exposure ceased. The findings confirm the prolonged storage of the highly-halogenated PCDF isomers and their preferential accumulation in the liver (Table 7-18). In a subject hospitalized for granulocytopenia, probably caused by long-term exposure to PCP, levels of highly chlorinated CDF and CDD were detected in adipose tissue (Gorski et al., 1984). The interest of this case consists in the two adipose tissue biopsies made at a 2-year interval. A rough estimate of the half-lives of these congeners has thus been suggested. Data reported in Table 7-19 confirm, although only preliminarily, that the half-lives of PCDF in humans exceed 1 year.

Schecter et al. (1985a,b) reported a high level of PCDF residues in adipose tissue of a patient who had been involved in the incident of the electrical transformer in the Binghampton State Office Building (BSOB) in NY. This transformer contained 65% PCB and 35% chlorinated benzenes. The soot samples contained a mixture of PCDFs and PCDDs in which the most toxic 2,3,7,8 substituted congeners were present. From Table 7-20 it appears that

TABLE 7-16

Levels of PCDD and PCDF in Blood Samples from Workers in the Saw Mill Industry After Exposure to 2,3,4,6-Tetrachlorophenolate[a]

Number	Profession	Chlorophenols in Urine (μg/mℓ)	PCDD (pg/g blood)				PCDF (pg/g blood)			
			octa	hepta[b]	hexa	penta	octa	hepta[c]	hexa	penta
Blank A		--	7	<2	<3	--	<3	2	<3	--
Blank B		--	<2	<1	<1	<1	<2	<1	<1	<1
1	Loader[c]	0.04	5	<2	<3	--	<3	40	<3	--
1	Loader[e]	5.2	7	<1	<1	<1	<2	22	<1	<1
2	Cleaner[d]	<0.02	5	2	<3	--	<3	30	<3	--
2	Cleaner[e]	0.23	22	8	<1	<1	<2	17	<1	5
3	Loader[d]	0.03	18	10	3	--	<3	18	<3	--
3	Loader[e]	0.83	3	<1	<1	<1	<2	17	<1	1
5	Packer[d]	<0.05	<3	<2	<3	--	<3	7	<3	--
5	Packer[e]	0.11	4	<1	<1	2	<2	12	<1	<1
7	Control	<0.01	3	<1	<1	<1	<2	3	<1	<1

[a] Source: Rappe and Buser, 1981

[b] Major isomer 1,2,3,4,6,7,8-HpCDD

[c] Major isomer 1,2,3,4,6,7,8-HpCDF

[d] Sampling 6 months after latest exposure

[e] Sampling after 1 month exposure

TABLE 7-17

Levels of PCDD and PCDF in Blood Samples from Workers in the Textile and Leather Industry After Exposure to Pentachlorophenol (PCP) or PCP Derivatives[a]

Number	Profession	Chlorophenols in Urine (μg/mℓ)	PCDD (pg/g blood)				PCDF (pg/g blood)			
			octa	hepta[b]	hexa	penta	octa	hepta[c]	hexa	penta
3	Textile	3.12	304	59	<1	<1	10	33	<1	<1
4		--	3	<1	<1	<1	<2	<1	<1	<1
5 M	Textile	<0.01	10	1	<1	<1	<2	<1	<1	10
6	Textile	0.42	105	15	<1	<1	<2	<1	<1	<1
7	Textile	0.16	30	6	<1	<1	<2	<1	<1	1
10	Tannery[d]	0.55[e]	20	7	<3	--	<3	7	<3	--
15	Tannery[d]	0.04[e]	80	30	3	--	7	18	3	--
16	Tannery[d]	0.03[e]	12	4	<3	--	<3	3	<3	--
17	Tannery[d]	--	7	2	<2	--	<3	3	<3	--

[a] Source: Rappe and Buser, 1981

[b] Major isomer 1,2,3,4,6,7,8-HpCDD

[c] Major isomer 1,2,3,4,6,7,8-HpCDF

[d] Blood sampling 8 months after last exposure

[e] Urine sampling 6 months after last exposure

TABLE 7-18

Levels of PCDF in Tissue Samples of a Worker Exposed to Phenoxy Herbicides[a]

Isomer	Concentration in[b]	
	Liver (pg/g organ)	Kidney (pg/g organ)
2,3,7,8-TCDF	<1	<1
2,3,4,7,8-PeCDF	10 (2)	7 (2)
1,2,3,4,7,8- + 1,2,3,6,7,8-HxCDF	55 (2)	8 (2)
1,2,3,4,6,7,8-HpCDF	100 (4)	6 (4)
OCDF	<3	<3

[a] Source: Rappe et al., 1983b

[b] The detection limits are in brackets

the isomers that were high in the patient's sample were also high in the soots to which the patient was exposed. Hence, in comparison to the levels of unexposed subjects, it would appear that these high levels arose through exposure to the soot. In this case the persistence of the 2,3,7,8 substituted isomers is noteworthy. On the other hand, the pattern of high levels of hexa- to octa- chlorinated dioxins in adipose tissue of both exposed and unexposed subjects is consistent with a more general environmental contamination.

Recent findings document the presence of PCDF and PCDD in the general population. Ryan et al. (1985a) reported the presence of 2,3,4,7,8-PeCDF in adipose tissues of Canadian patients at a level of 17 pg/g, confirming earlier data of Miyata et al. (1977a) who found the same isomer in tissues of controls in a study on Yusho rice oil poisoning. More recently Ryan et al (1985b) found 8-10 PCDDs and PCDFs, all with 2,3,7,8 chlorine substitution, in 46 adipose tissue samples from accident victims (in Canada). Major constituents were OCDD 800 pg/g), PeCDF, PeCDD, HxCDD and HxCDF. No TCDF and OCDF were detected. Levels ranged between 17 and 39 ppt, 2-3 orders lower than those associated with adverse effects in Yusho and Yu-Cheng patients. Similar patterns of congeners were found by Rappe et al. (1984) from samples of human Swedish control tissues. 2,3,7,8 Cl-substituted PCDFs have been detected in the general population from New York State by Schecter et al. (1985a,b) who compared tissues of subjects unexposed and exposed to chemical contamination (see Table 7-20). Schecter et al. (1986a) also found PCDD and PCDF at detectable levels in the general population of South and North Vietnam, areas of respectively high and low potential dioxin exposure. In both populations PCDFs were present, the North Vietnam samples showing levels

TABLE 7-19

Half-lives and Contents of PCDDs and PCDFs in Human Adipose Tissue[a]

Compound	Content in Adipose Tissue (ppb, on a fat basis) April 1981[b]	Content in Adipose Tissue (ppb, on a fat basis) November 1983[c]	Half-Life (years)
1,2,3,6,7,8-HpCDD	0.8	0.5	3.5
1,2,3,4,6,7,8-HpCDD	4.4	2.6	3.2
OCDD	5.2	4.0	3.7
1,2,3,4,6,7,8-HpCDF	0.8	ND	<1.7
OCDF	1.5	0.4	1.8

[a] Source: Gorski et al., 1984

[b] Determined by HR GLC-EC

[c] Determined by GLC-EC

ND = Not detected (<0.2 ppb)

TABLE 7-20

PCDD and PCDF Levels in Binghamton, New York Adipose Tissue Levels (ppt) in Exposed and Unexposed Subjects Soot Sample Levels (ppm) from the Transformer Fire[a]

PCDDs[b]	Soot	Exposed (n=1)	Unexposed (n=4)
2,3,7,8-TCDD	0.6	11.6	7.7
Other (4)	0.6		
1,2,3,7,8-PeCDD	2.5	15	10
Others (7)	2.5		
1,2,3,4,7,8-HxCDD	0.7		
1,2,3,6,7,8-HxCDD	0.6	72.6	55.4
1,2,3,7,8,9-HxCDD	0.4	7.3	7.3
1,2,3,4,6,8/1,2,4,6,7,9/1,2,4,6,8,9-HxCDD	1.2		
1,2,3,6,8,9/1,2,3,6,7,9-HxCDD	1.3		
1,2,3,4,6,7-HxCDD	0.5		
1,2,3,4,6,7,9-HpCDD	4.0	9.6	2
1,2,3,4,6,7,8-HpCDD	3.0	209	86
OCDD	2.0	690	602
TOTAL	19.9		

PCDFs[b]	Soot	Exposed (n=1)	Unexposed (n=4)
2,3,7,9-TCDF	12.0	ND	ND
1,3,7,9-TCDF	1.0		
Others	15.0		
1,2,3,7,8-PeCDF	310.0		
2,3,4,7,8-PeCDF	48.0	74.7	14.2
1,2,4,7,8-PeCDF	25.0		
1,2,4,7,9-PeCDF	22.0		
1,3,4,7,8-PeCDF	65.0		
2,3,4,8,9-PeCDF	25.0		
1,2,3,6,7-PeCDF	60.0		
2,3,4,6,7-PeCDF	12.0		
Others (12)	110.0		
1,2,3,4,7,8-HxCDF	310.0	149	14.2
1,2,3,6,7,8-HxCDF	150.0	112	8.9
2,3,4,6,7,8-HxCDF	10.0		
1,2,3,4,6,8-HxCDF	30.0		
1,2,3,6,8,9-HxCDF	38.0		
1,2,4,6,7,8-HxCDF	50.0		
1,3,4,6,7,8-HxCDF	125.0		
Others (8)	250.0		
1,2,3,4,6,7,8-HpCDF	230.0	39.3	13.6
1,2,3,4,6,7,9-HpCDF	120.0		
1,2,3,4,6,8,9-HpCDF	55.0		
1,2,3,4,7,8,9-HpCDF	55.0	25.9	10
OCDF	40.0	1.6	0.7
TOTAL	2168		

[a] Source: Buser, 1985; Schecter et al., 1985a,b

[b] Number in parentheses refer to the number of isomers

ND = Not detected

lower than in other more industrialized countries. Comparative data are reported in Table 7-21.

Further investigations prompted Ryan et al. (1985b) to seek information on the distribution and levels of PCDF in different tissues from the same subjects. From samples of two deceased elderly patients, they found that PCDF levels decreased from adipose tissue to liver, muscle and kidney in wet tissues, but the values were closer when calculated on the basis of lipid content. In a more complete study Ryan et al. (1986) showed that between 8 and 10 PCDDs and PCDFs with 2,3,7,8 chlorine substitution occur in all tissues tested, the relative amounts and specific isomers being the same (Table 7-22). Fat shows the highest levels and is probably a storage tissue but on a lipid basis, liver and other tissues have the highest levels, suggesting these may be target organs (Table 7-23).

Recently PCDDs and PCDFs have been detected in breast milk from human populations of different countries. Particularly high levels of 2,3,7,8-TCDD in milk of a South Vietnamese population were found in 1973 samples (Table 7-24) but in five 1983 breast milk samples the values were below the detection limits (Schecter et al., 1986a). However, milk of the general population also appears to contain highly chlorinated PCDDs and PCDFs (see Table 7-24).

7.3.4. Metabolic Fate. No satisfactory metabolic pathway for halogenated DF has yet been reported although the metabolism has been regarded as a route of detoxification for PCDF (Matthews and Birnbaum, 1983). The more readily metabolized PCDF have adjacent unsubstituted carbon atoms. Data is scant on the identification of metabolites of PCDF isomers; quantitative studies are lacking. One of the few published investigations on the metabolism of PCDF reports that in salmon, an unidentified hydroxylated metabo-

TABLE 7-21

Levels (pg/g) of PCDDs and PCDFs in Human Samples from Sweden, U.S.A. and Vietnam Subjects[a]

	Adipose Tissue[b]							
	Sweden [31]	Canada [10]		U.S.A. [6]	South [15]		North [9]	
	C	A	B	C	A	B	A	B
2,3,7,8-TCDD	3 (0-9)	10	10/10	6.4 (3.7-8.3)	27.9	12/15	ND	
1,2,3,7,8-PeCDD	10 (3-24)	13.2	10/10	9.7 (7.5-13.8)	15.4	15/15	3.8	1/9
1,2,3,4,7,8-HxCDD	ND							
1,2,3,6,7,8-HxCDD	15 (3-55)	90	10/10	57.8 (46.2-64.2)	99.8	15/15	11.4	6/9
1,2,3,7,8,9-HxCDD	4 (3-5)							
1,2,3,4,6,7,8-HpCDD	97 (12-380)	116	10/10	95.2 (30.4-119)	178	15/15	28.8	6/9
OCDD	414 (90-763)	611	10/10	585 (428-695)	1326	15/15	104	8/9
2,3,7,8-TCDF	4 (0.3-11)							
2,3,4,7,8-PeCDF	54 (9-84)	18.4	9/9	14.7 (10.9-17)	21	15/15	14.7	6/9
1,2,3,4,7,8-HxCDF	6 (1-15)	17.3	8/9					
1,2,3,6,7,8-HxCDF	5 (1-13)			28.7 (15.1-52.8)	58.3	15/15	13.3	7/9
2,3,4,6,7,8-HxCDF	2 (1-7)							
1,2,3,4,6,7,8-HpCDF	11 (1-49)	39.4	7/9	16.4 (12.5-23.8)	28.8	15/15	7	3/9
OCDF	4							

[a] Source: Nygren et al., 1986; Ryan et al., 1985b; Schecter et al., 1986a

[b] Numbers in brackets refer to number of samples

A = Mean of positives
B = Number of positives
C = Ranges

TABLE 7-22

2,3,7,8-Chlorine-Substituted PCDDs and PCDFs in Human Unexposed Subject*
(Values in pg/g on a wet tissue basis)

Compound	Fat		Adrenal	Bone Marrow	Liver	Muscle	Spleen	Kidney	Lung
	Abdominal	Subcutaneous							
2,3,7,8-TCDD	5.7	6.0	3.8	ND	ND	ND	ND	ND	ND
2,3,4,7,8-PeCDF	17	17	4.9	4.4	ND	1.1	ND	ND	ND
1,2,3,7,8-PeCDD	7.8	8.2	3.1	12	ND	1.2	11	ND	ND
1,2,3,4,7,8-/	52	22	8.5	9.4	4.2	2.1	ND	2.1	ND
1,2,3,6,7,8-PeCDF									
1,2,3,6,7,8-HxCDD	64	60	35	30	6.5	7.9	1.9	2.5	1.4
1,2,3,4,6,7,8-HpCDF	15	12	3.5	2.7	2.1	ND	ND	ND	ND
1,2,3,4,6,7,8-HpCDD	110	120	55	48	22	14	13	5.2	2.9
1,2,3,4,6,7,8,9-OCDD	680	700	600	540	220	170	46	31	21
% Lipid	75	75	28	28	6.0	9.0	1.8	3.0	2.2

*Source: Ryan et al., 1986

ND = Not detected

TABLE 7-23

Total PCDD and PCDF Levels (pg/g) in Tissue Samples from an Unexposed Subject*

Tissue	% Lipid	Total PCDDs		Total PCDFs	
		Wet Basis	Lipid Basis	Wet Basis	Lipid Basis
Fat, abdominal	75	870	1160	84	110
Fat, subcutaneous	75	890	1180	51	68
Adrenal	28	690	2440	17	60
Bone marrow	26	630	2440	17	63
Liver	6.0	250	4220	6.3	110
Muscle	9.0	190	2160	3.2	36
Spleen	1.8	72	3980	3.2	180
Kidney	3.0	39	1290	2.1	70
Lung	2.2	25	1150	--	--

*Source: Ryan et al., 1986

TABLE 7-24

Levels of PCDDs and PCDFs in Breast Milk

	Germany[a] n=53	Sweden[b] n=4	U.S.[c] n=1	South Vietnam 1973[c] n=5	South Vietnam 1983[b] n=4 (pooled)
2,3,7,8-TCDD	ND[d]	0.6	ND[e]	3.8 (2-6.5)	ND[f]
1,2,3,7,8-PeCDD	11.0 (<1-40)	6.5 (3.5-13.8)			7.0
Total HxCDDs	46.3 (9-168)	27.5 (17-35)			87.0
1,2,3,4,6,7,8-HpCDD	48.8 (11-174)	59.5 (28-86)			150.0
OCDD	142.8 (13-664)	302.0 (197-484)		2.5 (3.4-12)	754.0
2,3,7,8-TCDF	4.2 (<1-5)	4.2 (2.2-8)			94.0
1,2,3,7,8-PeCDF	2.0 (<1-7)	<1	ND[g]	0.28 (0.6-0.8)	ND[f]
2,3,4,7,8-PeCDF	21.1 (1-67)	21.3 (7-53)			21.0
Total HxCDFs	18.4 (3-60)	7.4 (6-18.3)			30.2
1,2,3,4,6,7,8-HpCDF	7.4 (<1-20)	7.4 (4.4-12)			23.0
OCDF	27.0 (<1-86)	3.2 (ND-11)	15		46.0

[a] Source: WHO, 1986
[b] Source: Nygren et al., 1986
[c] Source: Schecter et al., 1986a
[d] Below detection level (5 ppt)
[e] Below detection level (2 ppt)
[f] Below detection level (0.5 ppt)
[g] Below detection level (10 ppt)

lite is formed from 2,8-DCDF (Zitko et al., 1973). Veerkamp et al. (1981) reported investigations on the metabolism of various PCDFs in the rat. No metabolites were detected in urine, feces and tissues of rats receiving OCDF. Conversely, 2-MCDF, 2,8-DCDF and 2,3,8-TrCDF in rats form mono- and di-hydroxylated metabolites. Moreover, metabolites containing sulfur (sulfone, thioether) could originate from 2-MCDF and 2,8-DCDF. Hydroxylation can take place at various positions in the rings. Five monohydroxy derivatives have been found from 2,8-DBCF, the most important probably being 2-chloro-8-hydroxy-DF, that can originate from the 1,2 or 2,3 corresponding epoxide.

A molecular model developed by Veerkamp et al. (1983) predicted PCDF metabolites. In contrast to the corresponding PCDD in which hydroxylation takes place only in the 2 and 3 positions PCDFs can give rise to many products depending on the chlorine substitution pattern. Recently the metabolism of 2,3,7,8-TCDF was studied by Poiger et al. (1984) in the rat, unfortunately using a compound containing considerable amounts of other PCDFs. Bile was collected for 48 hours; no TCDF or other PCDFs were detected in the extract. From the 13 chlorinated metabolites present, which accounted for 8.6% of the total dose, four methoxylated compounds that probably originated from the corresponding hydroxylated species were present in the major amounts. They were identified as trichloromethoxy-DF, tetrachloromethoxy-DF and two trichlorodimethoxy-DF isomers. The remaining 9 compounds wee detected in traces and their origin (from the parent compound or impurities) remains unclear.

7.4. ACUTE, SUBACUTE AND CHRONIC TOXICITY

7.4.1. Acute Toxicity. PCDFs are all toxic and some are acutely toxic. The toxicity depends on the number of Cl substituents and on their position. The tetra-, pentaand hexaderivatives are the most toxic. The most acutely toxic PCDFs are the PCDF congeners substituted in the lateral 2, 3, 7 and 8 positions.

There is a long latent period between administration and the first signs of toxicity; the LD_{50} must be established 30 days after treatment. The main signs in monkeys and guinea pigs were severe weight loss, atrophy of the thymus and spleen, debilitation of the lymphatic system, hemorrhage of the adrenals and urinary bladder, and single-cell necrosis in the liver. Histological examination of tissues revealed loss of lymphoid cells in the thymic cortex and hyperplasia of epithelial cells in the renal pelvis, ureter and urinary gladder. These lesions, together with a lack of liver pathology, hypocellularity of bone marrow, seminiferous tubules, lymphoid elements in spleen and Peyer's patches, were similar to those described for guinea pigs given large doses of PCDD (McConnell et al., 1978b; McConnell and Moore, 1979).

The toxic effects of PCDFs are markedly species-dependent, as are those of PCDDs. The guinea pig is the most sensitive species followed by the monkey, rabbit, rat and mouse. In the guinea pig, the LD_{50} for 2,3,7,8- TCDF is between 5 and 10 µg/kg bw (McKinney and McConnell, 1981). The same authors showed that there was no significant different in acute toxicity (LD_{50}) between 2,3,7,8-TCDF, 2,3,4,7,8-PeCDF (< 10 µg/kg) and 2,3,7,8-TBDF, while the LD_{50} of 2,3,4,7,8-HxCDF was 120 µg/kg (Moore et al., 1979; McKinney and McConnell, 1981). The rat is quite resistant to the acute toxic effects of 2,3,7,8-TCDF. The monkey manifests intermediate sensitivity.

These differences depend on the species' ability to metabolize and excrete the parent compound. Since 2,3,7,8-TCDF has a whole-body halftime of <2 days in the rat, this species is quite resistant to the acute toxic effect. The guinea pig, on the other hand, is extremely sensitive (an LD_{50} between 5 and 10 μg/kg) because the compound has a wholebody half-time of at least 20 days. The monkey has intermediate sensitivity because the whole body half-time is ~8 days. The pathological effects of acute treatment with 2,3,7,8-TCDF in the rhesus monkey are summarized in Table 7-25. Table 7-26 sets out the LD_{50} for 2,3,7,8-TCDF in several animal species.

Mixtures of PCDFs were often used in toxicity studies. Mixtures of TrCDFs and TCDFs given orally to rabbits at doses of 0.4 and 1 mg/kg (Bauer et al., 1961) caused severe liver necrosis and often proved fatal.

7.4.2. Subacute Toxicity. The most complete data relate to 2,3,7,8-TCDF. Mice given 22 oral doses over 30 days of 30, 200, 300 μg/kg bw of 2,3,7,8-TCDF did not develop clinical signs of toxicity (Moore et al., 1979). Autopsy showed liver weights and the liver weight/bw ratio elevated, and the thymus weight/bw ratio was low.

When 2,3,7,8-TCDF was fed to chicks for 21 days at doses of 1 and 5 μg/kg bw/day, mortality was 16 and 100%, respectively (McKinney et al., 1976). 2,3,7,8-TCDF at 5 μg/kg bw/day affected the liver moderately and caused thymic involution and edema.

2,3,7,8-TCDF caused sickness and some deaths in two groups of three rhesus macaques, fed for 6 and 2 months with 5 and 50 μg/kg diet (corresponding to 5 and 50 ppb) (McNulty et al., 1981). The principal pathological changes were trophy or squamous metaplasia of the sebaceous glands, involution of the thymus and hypoplasia of the bone marrow. In animals that did

TABLE 7-25

Summary of Pathologic Effects of 2,3,7,8-TCDF in Rhesus Monkeys*

Tissue	Gross Effects	Microscopic Effects
Skin and adnexa	Facial edema, dry skin, loss of eyelashes, loss of finger and toe nails, streaks on ocular surface of eyelids, and occlusion of external ear canal	Hyperkeratosis; squamous metaplasia, cystic dilation of glands
Fat	Moderate to severe loss	None
Lymphoid	None	Thymic atrophy from lymphoid cell loss; moderate atrophy of lymphoid cells in spleen and lymph nodes
Liver	No effect	No lesions observed
Bile duct	Markedly thickened	Hypertrophy of biliary duct and gall bladder epithelium, increased mucous secretion
Intestine	Hyperemia, petechial hemorrhage, focal ulceration and mucosal cysts	Loss of parietal cells, increased mucous cells, microcystic dilatation of crypts
Clinical pathology		Anemia, low serum cholesterol, mild lymphopenia, neutrophilia, low serum protein and albumin, elevated SGOT, BUN

*Source: Moore et al., 1979

TABLE 7-26

Acute Toxicity of 2,3,7,8-TCDF in Several Animal Species

Animal	LD_{50} (µg/kg bw)	Interval Between Administration and Death (days)	Reference
Chicken	>1 <5	21	McKinney et al., 1976
Guinea pig	>5 <10	9-20	Moore et al., 1979
Rat	6000	ND	Moore, 1975
Monkey	1000	14-31	Moore et al., 1979
Mouse	>6000	ND	Moore, 1975

ND = No data

not die during the feeding, recovery was complete after 3 months of a diet free of 2,3,7,8-TCDF. It was suspected that the toxic effects of 2,3,7,8-TCDF might accumulate above a certain critical dose and might be similar whether the substance was administered acutely or ingested gradually over weeks or months.

Male guinea pigs given 2,3,7,8-TCDF in six or more weekly doses of 1 µg/kg bw showed irreversible toxicity when a body burden of ~5.6-6.6 µg/kg bw was attained, resulting in progressive weight loss and death (Decad et al., 1981b). A later paper from Ioannou et al. (1983) reported that a single dose of 4 µg/kg or multiple doses of 1 µg/kg for 4 weeks did not produce any observable toxicity by day 36; when animals were killed the authors estimated the body burden of these animals to be 2.1 and 2.8, respectively. A body burden two or three times higher resulted in the deaths of three of the four animals treated. These data indicate that, despite differences in individual sensitivity, PCDF toxicity appears only after a critical body burden is reached. Similar results were obtained by Luster et al. (1979) in female guinea pigs.

Inbred C57Bl/6N mice, treated i.p. with 2,3,4,7,8-PeCDF (30 µg/kg once a week for 6-12 weeks) developed histopathological signs in the liver, thymus and spleen. In an "unresponsive" strain, the changes in these organs were generally mild (Nagayama et al., 1985a).

Administration to rats of an unspecified mixture of PCDF (TCDFs, PeCDFs and HxCDFs) at a concentration of 10 ppm in the diet (corresponding to 0.5 mg/kg bw) for 4 weeks induced chloracne-like lesions on the ears, loss of body weight, low hematocrit and hemoglobin values, increased serum cholesterol and liver lipids, and low serum triglycerides. At 1 ppm in the diet these effects were less marked (Oishi et al., 1978).

A summary of the subacute toxic effects of PCDF in various animal species is provided in Table 7-27.

7.4.3. Chronic Toxicity. Planned studies of toxic effects of chronic treatment with PCDFs are lacking. McNulty et al. (1981) reported that 2,3,7,8-TCDF administered to three male rhesus macaques in the diet (5 ppb) for 6 months caused sickness and two deaths. The major pathological changes were atrophy or squamous metaplasia of the sebaceous glands, mucous metaplasia of the gastric mucosa, involution of the thymus and hypoplasia of the bone marrow. Animals that did not die during TCDF dosing recovered completely after 3 months of TCDF-free diet (see Section 7.4.2.). Because of the small number of animals employed, these findings are preliminary. However, it is apparent that some cumulative action does occur, and it may be reversible to some extent. Death in the monkeys was preceded by weight loss, anorexia and depression, but no specific chemical lesion was identified.

A study was made using monkeys fed a 20-week diet containing a mixture of PCBs, PCQs and PCDFs with congeners similar to those found in Yusho oil, or a mixture of PCBs or PCQs, or commercial PCB (Kanechlor 400). Only the first group developed hair loss, edema of the eyelids, acneform eruptions, dermal hyperkeratosis and pigmentation (Kunita et al., 1985). These data confirm PCDFs involvement in the Yusho disease.

7.4.4. Contribution of PCDF to the Toxicity of Industrial Products and Environmental Contaminants. PCDFs are released in the environment as contaminants of industrial products and must be considered as adventitious pollutants. Several studies have compared the toxicity of industrial products contaminated with PCDFs with that of pure active ingredients. Vos and Koeman (1970) found significant differences in the toxicities of three commer-

TABLE 7-27

Subacute Toxicity of PCDFs in Laboratory Animals

Compounds	Species/	Dose (µg/kg/day)	Mortality (%)	Route	Clinical Pathology	Reference
2,3,7,8-TCDF	chick	1 (21 days) 5 (21 days)	16 100	gavage	Edema; thymic involution; slight or no liver damage; no porphyria; no ALA induction	McKinney et al., 1976
2-isomers-TCDF 4-isomers-PeCDF 4-isomers-HxCDF	Sprague-Dawley rat	50 (28 days) 500 (28 days)	none none	1 ppm 10 ppm diet	Thymic involution; dermal lesions; hypercholesterolemia; hyperlipidemia; hemolytic anemia; thrombopenia	Oishi et al., 1978
2,3,7,8-TBDF 2,3,4,7,8-PCDF 2,3,7,8-TCDF	C57B1 mouse	30-300 (30 days)	none	gavage	Thymic involution; hepatomegaly	Moore et al., 1979
PCDFs	mouse	50 (10 weeks)	none	0.6 ppm diet	Dermal lesions; hyperkeratosis; dilated hair follicles with keratinous material; damaged hepatocytes	Nagayama et al., 1979
2,3,7,8-TCDF	adult rhesus	90 (6 months)	67	5 ppb	Atrophy or squamous metaplasia sebaceous glands, mucous metaplasia and hyperplasia of the gastric mucosa; induction of the thymus; hypoplasia of the bone marrow	McNulty et al., 1981
	macaque (3)	300 (2 months)	33	50 ppb diet		
2,3,7,8-TCDF	mature male guinea pig (4)	1 µg/kg bw week (6-7 weeks)	50	oral	Bald patches, dehydration, swelling around eyes, no hepatomegaly	Decad et al., 1981
2,3,7,8-TCDF	immature female guinea pig	1 µg/kg bw week (6 weeks)	30	oral intubation	Hepatomegaly and thymic atrophy	Luster et al., 1979

cial PCBs with Clophen A-60 and Phenoclor DP-6 causing the highest, and Aroclor 1260 the lowest mortality in chicks fed for 60 days diets containing 400 ppm of each product. Subcutaneous and abdominal edema and centrilobular liver necrosis were recorded only in chicks fed Clophen or Phenoclor. Hydropericardium was recorded in nearly all chicks fed Clophen or Phenoclor but only occasionally in chicks fed Aroclor. Chemical porphyria was attributed to a direct effect of the PCB. Chemical analyses revealed the presence of polar compounds in the 25% diethyl ether fraction of Clophen and Phenoclor. The polar fraction of Clophen was toxic in the chick embryo assay, and the differences in toxicity of the three PCB preparations were confirmed by this assay. Mass spectrometry analyses indicated the presence of tetra- and penta-CDF in the polar fraction of Clophen and Phenoclor (Vos et al., 1970).

Later, Vos and Notenboom-Ram (1972) compared the toxic effects in rabbits of Aroclor 1260 (which was shown to contain 1 μg PCDF/g) and of a pure PCB isomer, 2,4,5,2',4',5'-hexachlorobiphenyl. The rabbits were given 120 mg of Aroclor and the hexachlorobiphenyl dermally 5 times a week for 28 days. The animals receiving Aroclor 1260 showed more severe skin lesions, hyperplasia and hyperkeratosis of the follicular and epidermal epithelium than those receiving the pure hexachlorobiphenyl. Liver damage (subcapsular and zonal necrosis, hydropic degeneration, peripheral and perinuclear shift of cell organelles, focal cytoplasmic hyaline degeneration, proliferation of smooth endoplasmic reticulum) and increased fecal coproporphyrin levels were similar in both groups. From these studies, the probable contributions of PCDF and pure PCB to the toxicity of crude preparations were assessed (Table 7-28).

Goldstein et al. (1978a) showed that porphyria, cutaneous lesions, changes in liver enzymes and morphological changes in the liver were identical in female CD rats (Charles River) fed pure hexachlorobenzene contaminated with 4 ppm OCDF and decachlorobiphenyls for 4 months, indicating that these changes were produced by hexachlorobenzene rather than by the contaminants.

Kimbrough and Linder (1978) compared the toxic effects of purified and technical grade pentachlorophenol (PCP) in Sherman rats given dietary concentrations of up to 500 ppm of each compound for 8 months. At 500 ppm, technical PCP had severe effects on the livers of female rats, mainly causing degeneration of liver cells and bile duct proliferation. In male rats the alterations were less marked. At 100 ppm, similar but less pronounced effects were observed; only mild alterations were noted at 20 ppm. At 500 ppm, purified PCP in the diet caused slight enlargement of liver cells with occasional eosinophilic cytoplasmic inclusions; no alterations were observed in the livers of rats fed 100 and 20 ppm. These results suggest that most of the toxicity associated with feeding technical PCP to rats at these concentrations stems from toxic contaminants rather than from PCP itself. Technical PCP has been shown to be contaminated with PCDD and PCDF (Goldstein et al., 1978b) but the contributions of the various isomers to the toxic effects of PCPs have yet to be established.

The toxicity of a soot that contaminated an office building in Binghamton (NY) after a fire involving an electric service transformer and its dielectric fluids that was composed of Aroclor 1254 (65%) and chlorinated benzenes (35%) with some trace additives, was studied; the soot was found to contain PCDD (2.8 ppm) and 2,3,7,8-TCDF (124-273 ppm) (Smith et al., 1981) and a tentative estimate of the total amount of PCDF was in the range of

TABLE 7-28

Probable Contributions of PCDF in Aroclor 1260 and Pure 2,4,5,2',4',5'-Hexachlorobiphenyl to the Toxicity in Rabbits of PCBs Applied at 120 mg/50 cm²*

Dose	Chloracne	Edema	Liver Damage	Hepatic Porphyria
Aroclor (with PCDF)	++	++	++	-
PCB (PCDF-free)	+	-	+	++

*Source: Vos and Notenboom-Ram, 1972

0.5%. In guinea pigs the oral LD_{50} of the soot (0.75% aqueous solution) was 410 mg/kg with an equivalent of 0.5 and 20 μg/kg of 2,3,7,8-TCDD and TCDF, respectively. On the basis of the reported respective LD_{50} (Huff et al., 1980) the toxicity could not arise only from these halogenated congeners but probably came from other pollutants as well (Silkworth et al., 1982). After acute treatment with the soot serum, triglycerides were elevated; alkaline phosphatases decreased; body, thymus and kidney weight decreased; and hyperplasia of the pancreatic duct developed. Guinea pigs fed soot developed liver steatosis and smooth endoplasmic reticulum proliferation and mitochondrial ultrastructural alterations (Turner and Collins, 1983, 1985). Dermal application to the rabbit at a dose equivalent to 500 mg/kg for 24 hours caused no overt toxicity, although hepatic centrilobular hypertrophy was observed. For subchronic studies soot was administered to guinea pigs for 90 days, mixed into food at five dose levels between 0.2 and 231.5 ppm. Mortality reached 35% at 231.5 ppm and 30% at 46.3 ppm. No effect attributable to soot exposure was noted in animals receiving 0.2 ppm soot. Toxic effects were similar but rose at slightly lower total doses than with acute exposure (DeCaprio et al., 1983).

The i.p. toxicity in immature Wistar rats (100g) of a PCDF composition the same as that found in Yusho oil was compared (Bandiera et al., 1984a) with that of the corresponding PCB composition; 20% weight loss and 50% reduction in thymus weights relative to controls was caused by 398 mg PCB/kg bw. The PCDF mixture was as follows: 2,3,7,8-TCDF, 7.4%; 1,2,4,7,8-PeCDF, 6.1%; 1,2,3,7,8-PeCDF, 19%; 2,3,4,7,8-PeCDF, 29.4% and 1,2,3,4,7,8-HxCDF, 39.1%.

The PCQ contaminants in Yusho oil were as toxic as PCBs in eliciting Yusho symptoms (Hori et al., 1978, 1982). Thus, PCDFs are the most probable causal agents of Yusho. This was also shown (Kunita et al., 1985) explicity by experiments on rats and monkeys.

7.4.5. Some Particular Aspects of the Toxic Reactions.

7.4.5.1. DERMAL TOXICITY -- Chloracne can be produced by a number of chlorinated aromatics and has been observed among workers in contact with these compounds. Chlorinated naphthalenes, PCB, PCDD and PCDF can all induce chloracne in man. Chloracne is described as the formation of comedones with or without cysts and pustules. In chloracne the follicular orifices become clogged with sebaceous and keratinous material. Hyperkeratosis of the epidermis, cystic dilatation of the hair follicles, and an increase in melanin pigment are fairly common findings. Chloracne can be induced by external contact with these chemicals or by systemic absorption.

Investigations in animals have shown that different species develop different types of skin lesions on exposure to chloracnegenic compounds. Rabbit ear skin provides a good test for the development of acneform dermatitis. Application of a mixture of TrCDF and TCDF to the rabbit ear resulted in hyperplasia and hyperkeratosis (Bauer et al., 1961). Yos and Beems (1971) reported that application to rabbit skin of a 25% diethylether-extracted polar fraction of Phenoclor and Clophen (identified as consisting of TCDFs and PeCDFs) caused hyperplasia and hyperkeratosis of the follicular epithelium. The fraction from Phenoclor was more active than that from Clophen.

Vos and Notenboom-Ram (1972) compared the skin toxicity of Aroclor 1260 with 2,4,5,2',4',5'-hexa-CB. Dermal application of 120 mg PCB/50 cm^2 5 times a week for 4 weeks to the shaved backs of rabbits resulted in early macroscopic skin lesions in the Aroclor group, but not in the group given the pure PCB (see Table 7-28).

TCDFs and PeCDF induced hyperkeratosis of the skin when applied to rabbit pinna for 3 days. When daily doses of 4 μg of 2,3,7,8-TCDF were applied to the rabbit ear for 5 days, the animals developed hyperkeratosis of the treated ear (Kimbrough et al., 1978). Hyperkeratosis was also produced in hairless mice fed rice oil contaminated with Kanechlor 400 (Inagami and Koga, 1969). Ear lesions resembling chloracne were reported within 3 weeks in Sprague-Dawley rats fed a mixture of two tetra- , four PeCDFs, and four HxCDFs (Oishi et al., 1978) at a dose of 10 ppm PCDF in the diet and in mice fed 0.6 ppm PCDF (Nagayama et al., 1979). No acneigenic response in the rabbit ear bioassay was reported with 2,8-DCDF and 2,4,8-TrCDF (Kociba and Cabey, 1985).

The dermatological severity in Yu-Cheng patients 0.5 years after exposure was related (Kashimoto et al., 1985) to the presence of PCDF, PCQ and PCB in blood, but workers exposed to similar amounts of fresh PCBs showed only mild clinical signs. Investigations on 10 female Cynomolgus monkeys (Kunita et al., 1985) indicated that dermal symptoms characteristic of Yusho could be induced after 16-20 weeks by oral administration of PCDF from Kanechlor 400 (20 μg/day), or PCB (5 mg/day) plus PCDF (20 μg/day) but not by PCB alone (5 mg/day), or by PCQs (5 mg/day).

7.4.5.2. ULTRASTRUCTURAL LIVER ALTERATIONS -- A number of characteristic ultrastructural alterations in hepatic cells have been seen in rats and monkeys in response to dioxins and PCBs and in guinea pigs fed with BSOB soot (Kimbrough et al., 1972; Burse et al., 1974; Silkworth et al., 1982; Turner and Collins, 1985). They consisted of lipid droplet formation, increased smooth and rough endoplasmic reticulum and mitochondrial alterations. Mitochondrial lesions in animals seem to be reversible and tend to return to their usual structure with the passage of time.

In humans similar mitochondrial structural alterations have been reported after exposure to chlordecone (Guzelian et al., 1980), to PCDFs + PCB in the Yusho incident (Yamamoto et al., 1971; Hirayama et al., 1969), to PCB, PCDD, PCDF, naphthalenes and biphenylenes in the BSOB electrical transformer fire (Schecter et al., 1984).

From these studies mitochondrial alterations (mitochondrial pleomorphism, giant mitochondria, arrangement of the mitochondrial cristae parallel rather than perpendicular to the mitochondrial axis) seem the most specific ultrastructural lesions for humans exposed to PCB and furans, while changes in endoplasmic reticulum and intracytoplasmic lipid droplets are frequently seen after toxic damage following exposure to nonchlorinated hepatotoxic chemicals (Schecter et al., 1984). Therefore, ultrastructural analysis of livers, together with the analysis of fat biopsies for their chlorinated chemical levels, may provide a useful biological marker in evaluating human exposure to dioxin and related compounds (Schecter et al., 1985b). The role of PCDF in these ultrastructural morphological lesions remains to be clarified.

7.4.5.3. PORPHYRIA -- In birds and mammals, exposure to PCB and PCP causes changes in hepatic porphyrin synthesis, producing a form of hepatic porphyria similar to human prophyria cutanea tarda (Goldstein et al., 1973, 1975, 1977a, 1978a). Disturbances of porphyrin biosynthesis have been connected with an increase in the activity of the rate limiting enzyme, ALA synthetase (Goldstein et al., 1976). PCDFs have been shown not to cause hepatic porphyria and to induce only slight ALA synthetase activity in laboratory animals. Goldstein et al. (1976) found that 2,3,7,8-TCDF did not induce ALA synthetase in chicks after 21 daily doses of 1 μg/kg bw and did not produce porphyria even at the lethal dose of 5 μg/kg bw/day, whereas specific PCB produced both effects. In rats, a mixture of PCDF (2 TCDFs, 4 PeCDFs and 4 HxCDFs) at dietary concentrations of 10 and 100 ppm for 4 weeks only slightly induced ALA synthetase activity and slightly increased uroporphyrins (Oishi and Hiraga, 1978). 2,3,7,8-TCDF did not cause porphyrin accumulation in livers of mice up to 28 days after a dose as high as 4 mg/kg bw (Goldstein et al., 1974).

The lack of effect of PCDF on porphyrin accumulation in the liver had already been emphasized by Vos et al. (1970), who observed that three commercial 60% chlorinated PCB mixtures (Phenoclor DP-6, Chlophen 1-60, and Aroclor 1260) induced a similar degree and type of porphyria although the degree of contamination by PCDF varied for these formulations. Chlophen and Phenoclor contained TCDFs and PeCDFs in amounts responsible for other toxic effects. These findings suggest that PCDFs, at least in the experimental conditions tested, do not play an important role in the porphyrogenic effects of the industrial chemicals they contaminate.

Based on some common mechanism of action of PCDF with PCDD and other porphyrogenic chlorinated compounds, the hypothesis that PCDF can also elicit porphyria cannot be ruled out, although this has not yet been experimentally established.

7.4.5.4. IMMUNOSUPPRESSION -- Few studies have aimed at finding alterations of the immune system by PCDFs in laboratory animals. Toxic doses of 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF and 2,3,7,8-TBDF have been shown to cause severe thymic atrophy in the guinea pig and mouse (Moore et al., 1979). Lymphopenia, atrophy of the thymic cortex, and reduction in the number of germinal centers in the spleen appeared in chicks dosed with 1-5 μg/kg bw for 21 days (McKinney et al., 1976). Studies in the female guinea pig (Luster et al., 1979) showed that the effects of 2,3,7,8-TCDF on the immune system resemble those of 2,3,7,8-TCDD. Depression of cell-mediated responses was found only at higher dosages tested (0.5-1 μg/kg bw weekly for 6 weeks). In this animal species the LD_{50} is 5-10 μg/g. Humoral immunity was only slightly depressed. These results were interpreted by the authors as indicating possible marked effects of PCDFs on the immune system. As for 2,3,7,8-TCDD, consequences would be severe if 2,3,7,8-TCDF were administered during the developmental phase of the immune response.

Single doses of 2,3,7,8-TCDF have been tested in mice. Reduction in thymus weight and splenocyte numbers were observed with relatively low doses of 2,3,7,8-TCDF given either orally or i.p. (100 μg/kg bw) to C57Bl/6 mice, where the LD_{50} for 2,3,7,8-TCDF is reported to be >6000 μg/kg bw (Moore et al., 1979). Humoral antibody production was significantly inhibited with a dose-response curve similar to that of 2,3,7,8-TCDD, but 2,3,7,8-TCDF was ~30 times less active than 2,3,7,8-TCDD, the latter being as suppressive after 6 weeks as after 1 week, while recovery from the effects of 2,3,7,8-TCDF was complete by 6 weeks (Vecchi et al., 1983a).

The effects of a mixture of TCDD and TCDF on immune responses (thymus weight and humoral antibody production) were investigated using the toxins at different ratios (TCDD:TCDF = 1:1; 1:10; 1:100 μg/kg) to cover different "natural" conditions of exposures (Vecchi et al., 1985). TCDF given alone at each of the three doses used had no effect on thymus weight; added to the TCDD, it did not modify its effect on thymus weight. Work now in progress shows that an additive depressive effect appears at the 1:100 ratio in antibody production.

Increased susceptibility to endotoxin has been described in mice treated orally with a TCDF (12%) and PeCDF (88%) mixture once a week for 4 weeks (Oishi and Hiraga, 1980).

2,3,7,8-TCDF-induced thymic atrophy and immunosuppression were more marked in mouse strains sensitive to induction of AHH by 3-MC or by 2,3,7,8-TCDD than in strains genetically more resistant to induction, as described for TCDD (Vecchi et al., 1983b), suggesting that the individual genetic background may play a role in the degree of PCDD- and PCDF-induced immunotoxicity.

Recent data have shown a positive correlation between AHH inducing ability (see Section 7.4.6.) of different PCDFs given as single doses, and thymic involution (Yoshihara et al., 1981). Studying in vivo and in vitro structure-activity relationships, Mason et al. (1985) found a good linear correlation ($v = 0.88$) for 15 PCDF between thymic atrophy and in vitro AHH induction. Thus, toxicity of DFs to the immune system could be related in some way to their inducing potential.

Interestingly, there are also reports (Kunita et al., 1985; Hori et al., 1981) that, in female monkeys, commercial PCB preparations (Kanechlor 400) specifically treated to eliminate contaminant PCDF, given in the diet for 20 weeks, were more immunosuppressive on humoral antibody production than the commercial PCB with PCDF impurities or normal commercial PCB preparations. An antagonistic effect regarding immunosuppression and enzyme inducibility in mice was reported by Rizzardini et al. (1983) who administered to C57B16 mice an artificial mixture of 2,3,7,8-TCDD and 2,3,7,8-TCDF at the ratio of about 1:8 and found that the mixture elicited a significantly lower effect on antibody production (50% inhibition) than TCDD alone (80% inhibition). These data seem to be preliminary and need further investigation. Conversely, other reports suggest that the simultaneous presence of PCDF and PCDD in BSOB soot with the approximate composition of 1.2 ppm TCDD, 48 ppm TCDF and 0.5% PCB may have synergistic effects with regards to toxicity in guinea pigs (Silkworth et al., 1982).

Male Sprague-Dawley rats (Nakanishi et al., 1985a) given 1.14 mg PCDF/kg bw by gastric intubation 3 times/week or 6 times/2 weeks showed necrosis of the Clara cells in the lung bronchioles and marked atrophy of the thymus, whereas similar treatment with 11.4 mg Kanechlor 400/kg bw had only mild effects. Male Sprague-Dawley rats and female Cynomolgus monkeys (Kunita et al., 1985) given 67 μg PCDF from Kanechlor 400/kg bw and 8 μg PCDF/kg bw/day, respectively, over 22-day and 20-week study periods presented immunosuppression and thymic atrophy. The PCBs from Kanechlor 400 were much less potent than the PCDFs.

Recently Kochman et al. (1985) found, in human subjects exposed to PCDF as a consequence of a PCB transformer fire and of the dispersion of the soot in the environment, an increase in a specific subpopulation of T suppressor cells, although the total suppressor as well as the total helper cells were similar in control and in exposed subjects.

7.4.5.5. ENZYME INDUCTION --

7.4.5.5.1. Introduction -- The mechanism of toxicity of PCDFs has been extensively studied but has not yet been elucidated. These compounds are potent inducers of one of the hepatic mixed function oxidases, AHH, that metabolized precarcinogens such as benzopyrene. Investigations both *in vitro* and *in vivo* to clarify the biochemical basis of the inductive effect of TCDD and related polyhalogenated congeners have led to the identification of a macromolecular species in hepatic cytosol of rodents that binds these compounds specifically with high affinity (Poland et al., 1976). The cytosolic protein is known to act as a receptor for AHH inductive activity. No evidence is currently available of direct involvement of the induction of AHH with any sign of toxicity induced by these compounds; however, the binding to the _Ah_ receptor as a mediator of toxicity is now accepted from the data available on structure/activity studies and genetic segregation of toxicity with the _Ah_ locus (Roberts et al., 1985).

7.4.5.5.2. AHH Induction -- PCDFs are potent inducers of the hepatic microsomal n-monooxygenase system (Goldstein et al., 1978b; Poland et al., 1976; Kawano and Hiraga, 1978). This enzyme complex (also called hepatic microsomal drug-metabolizing enzymes) metabolizes drugs and other foreign compounds. It consists of NADPH-cytochrome P-450 reductase and cytochrome P-450, the terminal component that contains the active site of the enzyme and determines substrate specificity. There are several distinct isoenzymes of cytochrome P-450 in liver microsomes that have different substrate specificity and are induced differently by a number of compounds.

Compounds that induce hepatic microsomal monooxygenase activities can be divided into two major classes: one is typified by phenobarbital that induces several subspecies of cytochrome P-450 and associated monooxygenase activities directed toward a wide variety of substrates; the other is represented by 3-methylcholanthrene (3-MC) that induces distinct isoenzymes of cytochrome P-450 termed respectively in the rat cytochrome P-450C or P-450d (Thomas et al., 1983) and in the mouse P_1- and P_3-P450 (Nebert and Negishi, 1982). These isoenzymes are associated with enzyme activities for a more limited group of substrates and AHH activity is one of them. The PCDFs belong to the same class of inducers as 3-MC.

In the rat (female CD strain, Charles River), 2,3,7,8-TCDF (89% pure and 2% unspecified PeCDF) given for 3 days at doses of 0.1-2.5 μg/kg bw/day was a potent inducer of hepatic drug-metabolizing enzymes (Goldstein et al., 1978b). The approximate ED_{50} for AHH induction in the rat (0.5 μg/kg bw x 3 days) is roughly double that reported for 2,3,7,8-TCDD (as a single injection); which is 30,000 times more potent that 3-MC (Poland and Glover, 1974) 2,3,7,8-TCDF reduced N-demethylase activity at all doses (Goldstein et al., 1978b) and increased P-448 [Previous terminology for cytochrome P-450 isoenzymes induced by 3-MC was "cytochrome P-448." This term will be used in this paragraph to denote all forms of cytochromes induced by 3-MC class of inducers as simplified expression.] Kawano and Hiraga (1978) investigated the effect of a mixture of tetra- (12%) and penta-CDF (88%) in male Wistar JCL rats (105-165 g bw) at doses of 10, 100, 1000 μg/kg bw/day for 3 days. They found effects similar to those elicited by 3-MC (20 mg/kg bw) on microsomal drug-metabolizing enzymes. Both drugs increased p-nitroanisole demethylase activity, moderately increased aniline hydroxylase activity, and produced little change in aminopyrine demethylase activity. Furthermore, both drugs increased cytochrome P-448.

The AHH inducing potencies of PCDF, TCDD and PCB were compared in rats by Nagayama et al. (1983). A mixture of PCDF (13% 1,2,7,8-TCDF; 35% 2,3,7,8-TCDF; 1% 1,2,4,7,8-PeCDF; 49% 1,2,3,7,8-PeCDF; 1% 2,3,4,7,8-PeCDF; 1% unspecified hexa-CDF) and TCDD at the dose of 5 μg/kg bw and a PCB mixture (Kanechlor-500) at the dose of 50 mg/kg bw were given i.p. TCDD only enhanced AHH activity in the prostate, thymus and spleen. In the kidney, lung and liver the order of AHH inducibility was TCDD > PCDF >> PCB. PCDF caused the greatest induction in the lung. Thus, the lung and kidney seem more sensitive than the liver to the inductive properties of these compounds.

Recently, Bandiera et al. (1984a) treated rats with a reconstituted mixture of PCDF and PCB that reproduced the approximate composition of the PCDF and PCB persisting in the liver of Yusho patients according to the most recent data (Rappe et al., 1983a; Masuda and Kuroki, 1982; Nagayama et al., 1983). The mixture of PCDF, including 2,3,7,8-TCDF; 1,2,4,7,8-PeCDF; 1,2,3,7,8-PeCDF; 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF (7.4, 6.1, 19.0, 24.9 and 38.1%, respectively), was injected i.p. at various doses. The dose-response pattern on AHH induction showed that the mixture elicited, 4- and 40-fold increases, respectively, in the liver enzymatic activity of rats, at doses of 22 and 63 µg/kg bw. All the PCB mixtures were significantly less active as AHH inducers than PCDF. From an analysis of the PCB:PCDF ratios in the contaminated rice oils in Taiwan (100:0.32) and in Japan (100:0.16) and of the ratios of the respective EC_{50} for AHH induction (at least 1:700), the authors suggest that PCDF must be considered the major etiologic agent in the contaminated rice oils and in Yusho poisoning of victims still suffering from this syndrome (Bandiera et al., 1984a; Sawyer and Safe, 1985).

Induction of AHH activity by 3-MC is expressed almost exclusively as a single autosomal dominant trait regulated by the Ah locus (Nebert et al., 1975). In inbred strains of mice AHH inducibility is genetically separate. Eight PCDF were given 30 µg/kg i.p. to four inbred strains of mice with different phenotypes of Ah locus (Nagayama et al., 1985b). In responsive mice (C57) with the exception of 1,2,3,6,7-PeCDF and 1,2,3,4,6,7-HxCDF all the PCDFs were active; in unresponsive strains (DBA and DDD) no induction was elicited by the 8 PCDFs tested. Results indicate that AHH responsiveness in mice segregates with the AHH inductive activity by PCDF and may also segregate with the toxic potency of the isomers as in fact has been demonstrated for the immunotoxicity (Vecchi et al., 1983b).

Human tissue appear to have Ah receptor molecules with properties similar to those observed in animals (Nebert and Jensen, 1979; Roberts et al., 1985; Gasiewicz and Rucci, 1984). Recently Wong et al. (1985, 1986) conducted a study on pregnant Chinese women in Taiwan exposed 4-5 years earlier to contaminated rice oils containing PCDFs, PCBs and PCQs. Placental microsomal fractions from exposed subjects presented significantly high AHH and ethoxyresorufin-o-deethylase (EROD) activities. In the same subjects placental micromes were found to contain a protein that cross-reacted with the antibodies against cytochrome P-450, isoenzyme 6. This isoenzyme is known to be induced by polychlorinated hydrocarbons.

The effect of PCDF was studied in human lymphoblastoid cell lines with different AHH inducibility for 3-MC (Nagayama et al., 1985b). Degrees of the enzyme inducibilities of PCDFs increased proportionally with those for 3-MC, indicating that in humans also, AHH inducibility for PCDF reflects the genetic susceptibility of the cells. Induction with 2,3,4,7,8-PeCDF; 1,2,3,4,6,7-HxCDF and 1,2,3,4,7,8-HxCDF was comparable to that with TCDD and higher than with TCDF, indicating a species difference compared with the laboratory animals.

7.4.5.5.3. Structure-Activity Relationship for Induction -- The structure/activity relationship of PCDFs to AHH activity was studied using the chick embryo by Poland et al. (1976) who found that 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF, as well as the corresponding CDD derivatives, were the most active isomers. These studies showed that both the number and the position of the chlorine atoms are important. The isomers that induce AHH activity have two common properties: halogen atoms should occupy at least three, and for maximal potency, four of the lateral ring positions (positions 2, 3, 7 and 8); at least one ring peri-position (positions 1, 4, 6, 9) should be unsubstituted.

Nagayama et al. (1983) demonstrated in rat lung and liver microsomal preparations that chlorination at position 1 reduced AHH inducibility. In fact 2,3,7,8-TCDF and 2,3,4,7,8-PeCDF are more active on liver AHH induction than 1,2,3,7,8-PeCDF and 1,2,3,4,7,8-HxCDF. A similar reduction was observed for induction in the lung by 2,3,6,7-TCDF and 2,3,4,6,7-PeCDF in relation to 1,2,3,6,7-PeCDF and 1,2,3,4,6,7-HxCDF. Moreover, an additional Cl substitution at the C-4 (or C-6) position increases the AHH induction potency in relation to the corresponding C-1 or C-9 substitutes.

Several data suggest that species differences may change the order of PCDF isomers for AHH-inducing activity. In mice 2,3,7,8-TCDF elicited the highest AHH induction (Nagayama et al., 1985b,d), in rats 2,3,7,8-TCDF for some authors (Yoshihara et al., 1981; Nayayama et al., 1983) in liver and lung microsomes but 2,3,4,7,8-PeCDF for others (Bandiera et al., 1984b; Mason et al., 1985) in liver.

The small number of *in vivo* kinetic data available on this class of compounds make it difficult to compare *in vivo* and *in vitro* results. The role of tissue distribution, biotransformation and elimination of PCDFs should be carefully considered for each compound under study. Only 2,3,7,8- TCDF has been studied in some detail in this regard (Decad et al., 1981a,b; Birnbaum et al., 1980, 1981; Birnbaum, 1985).

The most extensive structure/activity relationship studies have been made using the highly (AHH) inducible rat hepatoma H-4-11-E cell lines (Bradlaw and Casterline, 1979; Bandiera et al., 1984a,b; Mason et al., 1985).

Mason et al. (1985) correlated the *in vitro* AHH-inducing potency of PCDFs with the thymic atrophy or body weight loss caused by each congener in rodents. Although this is an obvious oversimplification of the problem it may be accepted as a good model when a rapid indication of the potential toxicity of new congeners is needed. This point will be discussed in more detail in other Sections.

Bradlaw and Casterline (1979), working with rat hepatoma cell culture [as a rapid screen test for detecting minute (pg) amounts of certain classes of compounds, e.g. PCDFs, PCDDs and PCBs], found that AHH activity was induced by PCDF containing at least 3 or 4 lateral ring positions substituted by chlorine atoms in the ring positions at 2,3,7,8; congeners with two to fewer chlorine atoms in the lateral positions showed no inductive effect up to a dose of 5 or 10 mg/kg bw.

Table 7-29 summarizes the quantitative *in vitro* data reported by Bandiera et al. (1984b) and Mason et al. (1985) concerning the relationship between the different TCDFs in their AHHand EROD-inducing potency. Bandiera et al. (1984b) showed that the AHH inductive ratios of the 2,3,4/1,3,8, 2,6,7/1,3,8, and 2,3,4,6/1,2,4,8 pairs of PCDF isomers were 128, 7 and 9, respectively.

Figure 7-1 reports a plot of the -log EC_{50} values for *in vitro* AHH inducing potencies versus the -log ED_{50} values for body weight loss and thymic atrophy for a series of PCDF congeners, a PCDF mixture with a composition similar to that found in the liver of Yusho patients and TCDD. Statistical analysis of both plots showed an excellent correlation, corroborated by the fact that the compounds tested differed widely in both *in vivo* and *in vitro* biological and toxic potencies. 2,3,7,8-TCDF was the most active AHH inducer *in vitro* and the most powerful and toxic congener *in vivo*.

TABLE 7-29

Effects of Different PCDF Congeners on Rat Hepatic Cytosolic Receptor Binding Avidities, AHH and EROD Induction in Rat Hepatoma H-4-II E Cells[a]

PCDF Congeners	Receptor Binding Avidities (ED_{50}) M	Enzyme Induction Potencies (EC_{50})	
		AHH (M)[c]	EROD (M)[c]
Dibenzofuran	<10	ND	ND
2-	2.8×10^{-4}	ND	ND
3-	$4.2 \pm 0.6 \times 10^{-5}$	ND	ND
4-	$<10^{-3}$	1.0×10^{-5}	1.71×10^{-5}
2,3-	4.72×10^{-6}	2.19×10^{-6}	4.84×10^{-6}
2,6-	2.46×10^{-4}	6.17×10^{-5}	6.31×10^{-5}
2,8-	2.57×10^{-6}	3.95×10^{-5}	4.0×10^{-5}
1,3,6-	4.40×10^{-6}	2.53×10^{-6}	3.37×10^{-6}
1,3,8-	8.50×10^{-5}	1.94×10^{-5}	3.02×10^{-5}
2,3,4-	1.9×10^{-5}	1.51×10^{-7}	2.48×10^{-7}
2,3,8-	$1.0 \pm 0.1 \times 10^{-6}$	2.49×10^{-6}	1.56×10^{-6}
2,6,7-	4.5×10^{-7}	2.80×10^{-6}	3.13×10^{-6}
1,2,3,7-	1.12×10^{-7}	2.70×10^{-5}	6.30×10^{-5}
1,2,3,6-	3.54×10^{-7}	$>10^{-4}$	$>10^{-4}$
2,3,4,7-	2.51×10^{-8}	1.79×10^{-8}	1.48×10^{-8}
2,3,4,6-	3.5×10^{-7}	1.32×10^{-6}	1.13×10^{-6}
2,3,4,8-	2.0×10^{-7}	4.14×10^{-8}	3.76×10^{-8}
2,3,6,8-	2.2×10^{-7}	1.04×10^{-6}	7.79×10^{-7}
2,3,7,8-	$4.1 \pm 0.6 \times 10^{-8}$	3.91×10^{-9}	2.02×10^{-9}
1,2,4,8-	$>10^{-5}$	1.20×10^{-5}	9.26×10^{-5}
1,2,4,6,7-	6.77×10^{-5}	3.25×10^{-7}	3.48×10^{-7}
1,2,4,7,9-	2.0×10^{-5}	3.77×10^{-8}	3.84×10^{-8}
1,2,3,4,8-	1.2×10^{-7}	2.09×10^{-7}	1.63×10^{-7}
1,2,3,7,8-	$7.45 \pm 2.04 \times 10^{-8}$[d]	2.54×10^{-9}	3.06×10^{-9}
1,2,4,7,8-	1.3×10^{-6}	1.06×10^{-7}	1.48×10^{-7}
1,2,3,7,9-	3.98×10^{-7}	8.60×10^{-8}	8.60×10^{-8}

TABLE 7-29 (cont.)

PCDF Congeners	Receptor Binding Avidities (ED_{50}) M	Enzyme Induction Potencies (EC_{50})	
		AHH (M)[c]	EROD (M)[c]
1,2,4,6,8-	$3.09x10^{-6}$	$1.00x10^{-5}$	$1.20x10^{-5}$
1,3,4,7,8-	$2.00x10^{-7}$	$1.60x10^{-9}$	$1.40x10^{-9}$
2,3,4,7,8-	$1.50\pm0.1x10^{-8}$[d]	$2.56x10^{-10}$	$3.79x10^{-10}$
2,3,4,7,9-	$2.00x10^{-7}$	$7.90x10^{-9}$	$1.24x10^{-9}$
1,2,3,4,7,8-	$2.3x10^{-7}$	$3.56x10^{-10}$	$3.79x10^{-10}$
1,2,3,6,7,8-	$2.7\pm1.0x10^{-7}$[d]	$1.47x10^{-9}$	$1.24x10^{-9}$
1,2,4,6,7,8-	$8.3x10^{-6}$	$4.24x10^{-8}$	$2.93x10^{-8}$
2,3,4,6,7,8-	$4.7\pm0.4x10^{8}$[d]	$6.87x10^{-10}$	$5.75x10^{-10}$
2,3,7,8-TCDD	$1.0x10^{-8}$	$7.23x10^{-11}$	$1.85x10^{-10}$

[a] Source: Bandiera et al., 1984b; Mason et al., 1985

[b] AHH = Aryl hydrocarbon hydroxylase

[c] EROD = Ethoxyresorufin o-deethylase

[d] The dose-response competition experiments were carried out in triplicate and illustrate the reproducibility of the assay with the PCDF.

ND = Not detected

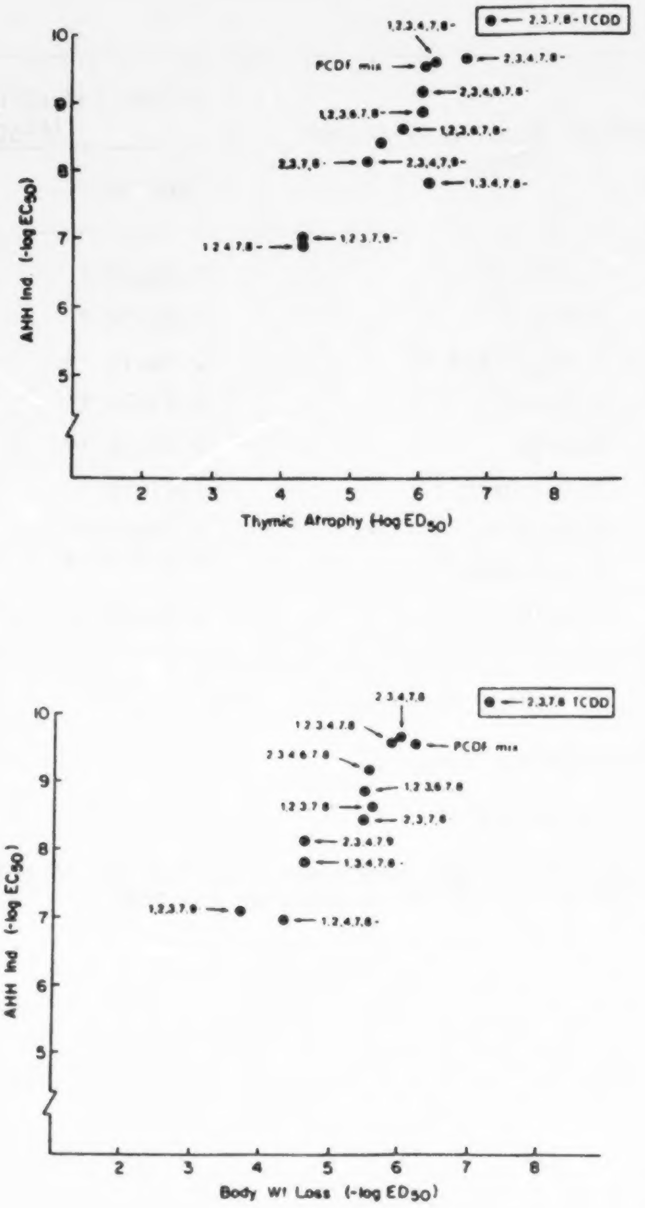

FIGURE 7-1

A plot of the -log EC_{50} values for AHH induction in rat hepatoma H-4-IIE cells vs. the _in vivo_ -log ED_{50} values for thymic atrophy (top) and body weight loss (bottom) for several PCDFs, 2,3,7,8-TCDD and a reconstituted PCDF mixture

Source: Mason et al., 1985

In human lymphoblastoid cells Nagayama et al. (1985c) showed that chlorination in position 1 has an activating effect. In these cells the HxCDFs and PeCDFs (1,2,3,4,7,8-, 1,2,3,4,6,7- and 2,3,4,7,8-) were more potent than TCDF and a sex effect appeared, with females consistently presenting higher inducibility. As far as species differences in AHH inducing activity are concerned, in rats and mice PCDFs were always less active than TCDDs but in human cells the most active isomers were as potent as TCDDs (Nagayama et al., 1985c). This must be taken into account in relation to their reported high persistence in tissue of humans (Rappe et al., 1983a) and undomesticated animals in evaluating their toxic potency.

A structure-activity relationship similar to that on AHH induction was reported by Nagata et al. (1981) for the effect of PCDFs on the microsomal metabolism of progesterone. Both 2,3,7,8-TCDF and 2,3,4,7,8-PeCDFs selectively increased 7α-hydroxylation, but strongly suppressed 2α, 6β and 16α hydroxylation and 5α reduction of progesterone. This change in the progesterone metabolic pattern results in a marked depression of total metabolism of the steroids. 2,8-DCDF; 1,3,6,7-TCDF; 1,3,6,8-TCDF and 1,2,4,6,8-PeCDF do not affect progesterone metabolism.

7.4.5.5.4. AHH Induction and Cytosol Receptor Binding -- The significance of the induction of drug-metabolizing enzymes by PCDFs and chlorinated aromatic hydrocarbons in general in the interpretation of their toxicity is still an open question (Poland et al., 1979; Yoshimura et al., 1979; Poland and Kende, 1976). An important observation was the correlation between AHH induction and binding affinities for the hepatic cytosol receptor protein of a certain number of PCDF congeners (Nagayama et al., 1983; Poland et al., 1979; Yoshihara et al., 1981; Bradlaw and Casterline, 1979). Recently the effect of structure on rat hepatic cytosol receptor affinity and AHH induction potency was studied for 26 pure PCDFs (Bandiera et al., 1984b) (see Table 7-29) that were synthesized according to a new reported procedure (Safe and Safe, 1984). As for AHH induction, the most active PCDFs for receptor binding were substituted in all four lateral positions. Moreover, compounds with additional Cl groups at C-4 or C-6 bind with higher affinity to the receptor than isomers with substituents at C-1 or C-9. However, there is no linear correlation between the receptor binding affinity and AHH induction potencies for the 26 isomers. This suggests that after initial binding of PCDF ligands with the receptor some molecular features (such as conformation of the ligand-receptor complex, differential ligand/receptor-nuclear interactions) distinct from those required for attachment, affect events in the complex enzyme induction process.

Structure/activity relationship (SAR) studies have been recently conducted (Mason et al., 1985) with a number of pure PCDFs and a mixture of PCDFs resembling the compositions of the mixture persisting in Yusho patients (Bandiera et al., 1984a) in an attempt to correlate their AHH inductive properties and toxic effects (thymic atrophy and weight loss). Results indicated an excellent linear correlation between in vitro and in vivo activity for the congeners that are substituted in both phenyl rings and do not contain vicinal unsubstituted carbon atoms. The effect of chlorine substitution at the four different positions of the dibenzofuran ring can be summarized as follows: 3,7 > 2,8 > 44,6 > 1,9. This differs from PCDDs, in which all the lateral positions are equivalent because of the symmetry of the dibenzo-p-dioxin ring system. Inspection of these results and of the suggested hypothetical structural features indicate that a molecule can be hypothesized with high affinity for the receptor but scant inductive power, thus eliciting no toxic effects. Such a molecule might be an antidote for the most toxic PCDF.

Keys et al. (1986) have recently reported that two TCDF congeners 2,4,6,8-TCDF and 1,3,6,8-TCDF possess the peculiarity of being poor *in vitro* inducers of AHH and EROD activities but have relatively high binding avidities to the cytosolic receptor protein. These compounds competitively displaced TCDD from the receptor and their coadministration with TCDD was significantly was reduced in the expected additive induction of AHH and EROD activities. On the other hand, 2,3,7,8-TCDF administered together with 2,3,7,8-TCDD gave additive enzyme induction responses.

Cheney (1982) and Cheney and Tolly (1979) developed a theoretical model suggesting that four structural factors, including the fused tricyclic aromatic system, were important for the attachment and stimulation of AHH induction, while the electronegative substituent of the lateral ring did not influence induction and binding in the same manner. More precisely, the latter appears to increase induction to an extent that is greatly out of proportion to its effect on binding. The lateral ring substitution seems particularly important to switch the receptor-complex to an active state to promote AHH induction.

In conclusion, the toxicity of PCDFs seems to correlate well with their potential capacity to induce AHH; however, toxicity is not bound directly to AHH activity, but both AHH induction and toxic effects are to be considered secondary to the initial binding to the cytosolic protein receptor.

In the last 2 years a link has been found between TCDD, which is the most powerful and the most widely studied of the polychlorinated hydrocarbons, and the regulation of epidermal growth factor (EGF) binding in human keratinocyte lines was demonstrated (Hudson et al., 1985, 1986; Osborn and

Greenlee, 1985; Greenlee et al., 1985a). These authors demonstrated that TCDD can modulate the proliferation and differentiation of epidermal keratinocytes both in vivo and in vitro. This was explained by the competition of TCDD with EGF in binding to the Ah receptor present in basal cells.

Another important example of the relationship between the binding to a specific receptor and manifestation of a toxic effect was reported by Greenlee et al. (1985b) who showed that TCDD-induced thymic atrophy was mediated by a receptor protein in thymic epithelial cells. The major consequence of this fact appears to be altered thymus-dependent maturation of T-lymphocyte precursors, mediated through direct cell-cell contact between thymocytes and thymic epithelial cells.

7.5. REPRODUCTIVE/DEVELOPMENTAL TOXICITY

7.5.1. Introduction. The literature base on the potential reproductive and developmental toxicity of the PCDFs is limited, and prior assessments have relied largely on the similarity of these agents with the dioxins (U.S. EPA, 1983, 1984a). Recently, however, several laboratories have conducted studies that focus on the dibenzofurans. This chapter will pay particular attention to these studies, as well as summarize those studies that have been reviewed in previous U.S. EPA documents. In general, the greatest focus has been on prenatal development, with little information on other aspects of the reproductive process. Most laboratory studies have concentrated on 2,3,7,8-TCDF, since the TCDD homolog is known to be highly toxic and teratogenic.

7.5.2. Development. The studies by Nagayama et al. (1980) indicate that polychlorinated dibenzofurans (PCDFs) are capable of being transferred to the developing mouse, both prenatally through the placenta and postnatally through the breast milk. Exposure of the dams to a mixture of tetra-, penta- and hexachlorodibenzofurans was through the diet during the first 18 days of pregnancy or during the first 2 weeks of postnatal (PN) life. Low, but measurable, levels of PCDFs were found both in fetuses at gestation day 18 and in neonates at 1 and 2 weeks of PN life. Whole body concentrations were highest in the neonates, with the percentage of total intake at PN week 2 double those at PN week 1, suggesting an accumulation of the PCDFs during the early postnatal period. Weber and Birnbaum (1985) also examined the transplacental transfer of radioactive 2,3,7,8-TCDF in the C57Bl/6N mouse. Exposure was by gavage on gestation day 11 at a dose known to cause a high level of cleft palate. Low levels of radioactivity were found in the embryos at gestation day 12, but radioactivity in the embryos was below detectable limits by gestation days 13 and 14. Together, these two studies indicate that the PCDFs are transferred at low levels to the embryo and fetus transplacentally, and at higher levels to the nursing neonatal animal during early PN life.

Weber et al. (1984) studied the effect on prenatal development of single and multiple exposures to 2,3,7,8-TCDF in C57Bl/6N mice. The 2,3,7,8-TCDF was 98% pure (primary contaminant was PeCDF). Administration was by oral gavage in corn oil (10 mℓ/kg/treatment). Single exposure was on gestation day 10 (plug day = gestation day 0) at 0 (vehicle control), 250, 500 and 1000 μg 2,3,7,8-TCDF/kg bw. Multiple exposures were on gestation days 10-13, with daily exposure levels at 0, 10, 30, 50 and 100 μg 2,3,7,8-TCDF/kg bw. The maternal animals were monitored for body weight changes and signs of toxicity during gestation, and the uterine contents were examined on gestation day 18 before parturition. The fetuses were examined for gross and visceral malformations, but were not examined for skeletal anomalies. There were no signs of maternal toxicity, except for changes in absolute and relative liver weight at the highest dose under both exposure regimens. The authors indicate that this is probably a metabolic response of the liver to 2,3,7,8-TCDF exposure, although they note that a toxic effect on the liver cannot be ruled out.

In terms of developmental toxicity, the most significant effects were on the kidney and palate. Following single exposure, there was a significant, dose-related increase in litters with hydronephrosis at all doses (250, 500 and 1000 µg/kg) and litters with cleft palate at the highest dose (1000 µg/kg). Following multiple exposure a similar pattern was observed, with an increase in litters with hydronephrosis starting at 30 µg/kg/day and an increase in litters with cleft palate starting at 50 µg/kg/day. The authors also reported an increase in fetal mortality above control levels at all doses following single exposure (0% in control; 12.6% at 250; 14.7% at 500; 21.1% at 1000 µg/kg). However, even though this was statistically significant, the finding must be viewed with caution, since a total absence of fetal mortality in mice, even in the control population, is uncommon. For example, the control group for the multiple exposure design in this study (Weber et al., 1984), as well as a control group in another study by this laboratory (Weber et al., 1985), exhibited 11.7% and 10.5% fetal mortality, respectively, at a level comparable to the 2,3,7,8-TCDF-exposed groups. There were no other gross or visceral malformations observed and there was no effect on fetal weight. The authors do not indicate why the TCDFs would not be expected to produce skeletal changes. However, the exclusion of this major class of anomalies, which is often quite sensitive to insult, may have limited the effects that were seen.

These results demonstrate that 2,3,7,8-TCDF is a developmental toxicant in the mouse at exposure levels that are not maternally toxic. Based on the litter, which is generally considered the experimental unit in this type of study design, the LOAEL was at 250 μg/kg/day following a single exposure and 30 μg/kg/day following the 4-day exposure. A NOEL was not established for a single exposure; for multiple exposures the NOEL was 10 μg/kg/day. Several additional points should be recognized in analyzing the data. First, the number of animals/dose group was low. The current U.S. EPA recommendation is 20 litters/dose group (U.S. EPA, 1984b); however, in this study, the number of litters ranged between 6 and 11. This does not reduce the importance of the observations, but had the number been increased the power of the study to detect change may have been increased, with effects being seen at lower doses. Second, while the litter is considered the experimental unit in this study design, the individual fetal data should not be discounted. Kidney changes as a mean percentage of fetuses were significantly increased following multiple exposure at the 10 μg/kg/day dose. Since these changes were consistent with the dose-related response of the other endpoints on both a litter and individual fetus basis, it is possible that 10 μg/kg/day would be the LOAEL following multiple exposure. Third, hydronephrosis can be a reversible alteration in development; and the authors note that preliminary studies suggest this may be the case for the kidney changes observed following TCDF. However, without information on the level and duration of exposure and the postnatal age at observation, it is not possible to determine the significance of these findings relative to the developmental toxicity of the agent. Moore et al. (1973) examined the effect of pre- and postnatal exposure combinations to TCDD on the postnatal observation of kidney lesions and concluded that the incidence of hydronephrosis is probably a function of dose and length of exposure. Moreover, the developmental significance of transient changes is not well-understood but should not be arbitrarily discounted. Thus, these three points should be kept in mind in the overall assessment of the toxicity of the PCDFs, as they may influence the final determination of the effect levels.

Recently, Birnbaum et al. (1986) have reported, in abstract form, preliminary data from their studies on the relative teratogenicity of three PCDFs. C57Bl/6N pregnant mice were exposed to 2,3,4,7,8-PeCDF; 1,2,3,7,8-PeCDF; or 1,2,3,4,7,8-HxCDF on gestation days 10-13 and examined for teratogenic effects on gestation day 18. All three PCDFs were teratogenic, as evidenced by the occurrence of cleft palate at doses that did not produce maternal toxicity. The ED_{50}s were 40, 100 and 400 μg/kg for 2,3,4,7,8-PeCDF, 1,2,3,7,8-PeCDF and 1,2,3,4,7,8-HxCDF, respectively. The NOEL for 2,3,4,7,8-penta-CDF was 1 μg/kg, apparently lower than that for the 2,3,7,8-TCDF. Hydronephrosis occurred at even lower doses. Further analysis of this study will be done as the information becomes available since the apparent increased toxicity of 2,3,4,7,8-PeCDF may have an effect on the reported NOEL.

Weber et al. (1985) reported on the teratogenic potency of TCDF and TCDD when exposure to these two agents is combined. Individually, each agent showed a steep dose-response curve for the induction of cleft palate, with TCDD ~30 times more potent than TCDF. In combination, the two agents were additive in their contribution to cleft palate induction, with no indication of a synergistic or antagonistic effect of combined exposure.

Hassoun et al. (1984) also examined the developmental effects of 2,3,7,8-TCDF in mice of the C57Bl strain, using an i.p. route of exposure. Purity of the agent was 90%; 8% was hexachloroterphenyls, which the authors indicate "are probably less toxic and teratogenic" than TCDF. Exposure was by a single injection on gestation days 10, 11, 12 or 13 to the dioxane vehicle or TCDF at concentrations ranging from 0.1-0.8 mg/kg bw. The animals were examined on gestation days 16 or 17 for implantation sites, resorptions, and live and dead fetuses. Fetuses were examined for cleft palate and hydronephrosis. The number of treated dams in each dose group ranged from 5-14 (average = 8.8), which is less than optimal. Furthermore, the use of the i.p. route is generally not recommended since a mechanical or acute chemical insult can occur in the vicinity of the uterus. Nevertheless, this study supports the findings of Weber et al. (1984) and indicates that the most sensitive exposure period during gestation for the induction of cleft palate and hydronephrosis is gestation days 11-12.

In relation to human exposure to the PCDFs, Kuratsune (1976, 1980) has reviewed epidemiologic studies on Yusho (oil sickness), the incident in Japan in which humans were exposed to rice oil contaminated with PCBs containing a high level of PCDFs and other organochlorine compounds. Of 13 women who were exposed during pregnancy (11 showing signs of Yusho; 2 with husbands showing signs), 9 offspring had unusual grayish/dark brown pigmentation of the skin, gingivae and nails, and most showed a discharge from the eyes. Since similar signs were almost never seen elsewhere in Japan, the findings suggest an association between prenatal exposure and adverse outcome at birth. However, Kuratsune (1976, 1980) pointed out that the low number of cases does not allow for a clear cause and effect relationship to be drawn. Kuratsune (1976, 1980) also noted that 12 of the 13 babies were small by national standards, but this difference disappeared with age and the children had no apparent physical or mental handicap. In relation to postnatal exposure, Kuratsune (1976, 1980) summarized a report suggesting that boys of school age exposed to the contaminated oil showed a significant decrease in both height and weight relative to unexposed boys. Exposed girls did not show any such decrease relative to unexposed girls. Finally, from one case report, a child exposed only by nursing from the exposed mother began showing signs of Yusho, indicating transfer of the toxic agents through the breast milk.

7.5.3. Reproductive Function/Fertility. Studies designed to monitor the effect of the dibenzofurans on male and female reproductive function and fertility, apart from the developmental toxicity studies noted above, are minimal. Oishi et al. (1978) compared the toxicity of a mixture of TCDFs, PeCDFs and HeCDFS with that of a commercial polychlorinated biphenyl mixture. Male rats (100-130 g) were exposed through the diet to 0, 1 or 10 ppm PCDF for 4 weeks. In relation to the reproductive system, they found that the relative testes weight was increased at both the 1 and 10 ppm doses. There were other signs of general toxicity at these doses, including decreased body weight and food consumption, and it is likely that the effect on relative testes weight was associated with these changes. At 10 ppm, there were reductions in the absolute and relative weights of the seminal vesicles and ventral prostates, as well as a decrease in the concentration of testicular testosterone. However, because of the other general and systemic toxicity seen at this dose level, it is not possible to determine if PCDFs had a direct effect on the male reproductive system. In reviewing the Yusho incident, Kuratsune (1980) noted a report indicating that irregular menstrual cycles and abnormal patterns of basal body temperature were observed in 60 and 85% of the exposed women patients, respectively. Unfortunately, other reproductive effects of the PCDFs must be assumed from studies on similar agents such as dioxin. A recent Health Assessment Document for Polychlorinated Dibenzo-p-Dioxin (U.S. EPA, 1985) has summarized this literature and it will not be repeated here. However, in the few studies that have examined reproductive endpoints not associated with developmental toxicity, there has been no clear indication of an adverse effect of the dioxins on reproduction.

7.5.4. **Summary.** The data clearly support the developmental toxicity of 2,8,7,8-tetrachlorodibenzofuran in the C57Bl/6N mouse at relatively low levels of exposure. The study of Weber et al. (1984) provides the most direct support of this toxicity, demonstrating an adverse effect following a single exposure to 250 μg/kg on gestation day 10 and following a multiple exposure to 30 μg/kg/day on gestation days 10-14. A NOEL could not be determined from the results following a single exposure. Following multiple exposure, no effect was observed at 10 μg/kg/day when the data were analyzed on a litter basis. Considering the additional points that are noted above on study design and data interpretation, it is possible that biologically significant changes would occur at even lower levels of exposure.

The potential human developmental toxicity of the PCDFs has not been systematically studied. However, the findings associated with the Yusho incident are suggestive that the PCDFs can have an effect on the fetus and on the developing child.

Studies on other reproductive effects associated with PCDF exposure do not provide sufficient data to be more than suggestive. Effects have been reported on specific aspects of both the male and female systems. However, additional study would be required to clearly delineate any possible direct effect of the PCDFs on reproductive function and fertility.

As noted in Section 7.5.1., the literature base on the chlorinated dibenzofurans in the area of reproductive and developmental toxicity is very limited. Many laboratories have focused on the dioxins, with a considerable extension of that data base, at the expense of the dibenzofurans. Since the data base on the dioxins is more extensive, it would be useful to continue and expand reviews that address the similarities and differences in the action of these two classes of agents. More directly, however, studies should be designed to more adequately assess the low end of the PCDF effect range relative to developmental toxicity, and studies on fertility and reproductive function should be initiated. In addition, a clearer picture should be developed regarding the effect of chemical species (since the majority of studies have examined 2,8,7,8-TCDF) and animal species and strain (since most studies have used the C57Bl/6N mouse). Finally, a better understanding of the pharmacokinetics and metabolism of these agents, especially as related to transplacental transfer, transfer through the breast milk, and localization/duration of exposure in the embryo/fetus and neonate would help in establishing appropriate experimental models and extrapolating data from animal to the human condition.

7.6. MUTAGENICITY

Schoeny (1982) tested four chlorinated dibenzofurans (2,8-DCDF, 3,6-DCDF, 2,3,7,8-TCDF, and OCDF) in the Salmonella histidine reversion assay. Four doses (0.1-10 µg/plate) of 2,8-DCDF were assayed in Salmonella strains TA1535, TA100, TA1975, TA92, TA2322, TA1537, TS24, TA2637, TA1538, TA98 and TA1978. 3,6-DCDF was tested identically except that strain TA1538 was not used. Five doses (0.1-20 µg/plate) of 2,3,7,8-TCDF were assayed in strains TA1535, TA100, TA1537, TA98 and TA1978; three doses (0.1-5 µg/plate) of octachlorodibenzofuran were tested in strains TA100, TA1975, TA92, TA2322, TS24, TA1537 TA2637, TA98 and TA1978. All four PCDFs were tested in the absence or presence of Aroclor-induced rat S9 mix. 2,3,7,8-Tetrachlorodibenzofuran was also tested in the presence of S9 mix from rats induced with 3-methylcholanthrene, phenobarbital and 2,3,7,8-TCDF; OCDF was also tested in the presence of S9 mix from rats induced with 2,3,7,8-TCDF.

The results were negative (mutagenicity was reported as none). Toxicity was not observed except for 2,3,7,8-TCDF at 1 μg/plate in the absence of S9 mix. Schoeny (1982) commented that the Salmonella histidine reversion assay is relatively insensitive to chlorinated hydrocarbons, perhaps because the S9 preparations cannot effect dechlorination or some other reaction necessary to produce active metabolites.

2,3,7,8-TCDF was also studied (along with several chlorophenols and chlorophenol impurities) in Saccharomyces cerevisiae strain MP-1 and was negative for forward mutation, mitotic crossing over and mitotic gene conversion at concentrations up to 1.0 mg/mℓ (Fahrig et al, 1978). Survival was 91% at 1.0 mg/mℓ. Stationary phase cells were tested in the absence of exogenous activation. The control values varied widely among the tests of the individual chemicals.

In summary, two studies (both negative) on the mutagenic potential of chlorinated dibenzofurans have been reported. Additional studies are needed before any conclusion can be reached concerning the mutagenicity of these compounds.

7.7. CARCINOGENICITY

7.7.1. Animal. Close structural similarity of PCDFs to PCDDs, especially 2,3,7,8-TCDD, which is a proven animal carcinogen, raises concern as to the potential carcinogenicity of 2,3,7,8-TCDF. However, no animal carcinogenicity bioassay data on PCDFs are currently available in the literature.

However, as PCDFs are contaminants of commercial PCB and might have contributed to the toxic effect in animals and man (Oishi et al., 1978; Vos et al., 1970; Cordle et al., 1978), it can be hypothesized that they play a role (e.g., promotion, cocarcinogenesis, initiation) in the suspected carcinogenic effect of PCB.

PCB mixtures (Aroclor 1254 and 1260, Kanechlor 400 and 500) reportedly produce hepatic tumors in mice and rats (WHO, 1978b; Kimbrough, 1974; Kimbrough et al., 1972). Recently the hepatocarcinogenic potential of Aroclor 1260 was confirmed in Sprague-Dawley rats (dose of 100 μg/g in the diet for 16 months), a strain with a low incidence of spontaneous hepatocellular neoplasms (Norback and Weltman, 1985). After 18 months, in the PCB-exposed group hepatocellular neoplasms were present in 95% of the 47 females and in 15% of the 46 males with a significant sex difference. In 81 controls only one hepatocellular neoplasm occurred. The appearance of tumors in the liver and stomach that do not occur spontaneously in control rats was also reported after treatment with Aroclor 1254 (25-100 ppm in the diet) suggesting that PCBs initiate lesions de novo rather than promote the development of naturally-occurring lesions (Ward, 1985).

7.7.2. Human. Amano et al. (1984) recently completed a 16-year cohort mortality study of 1086 Yusho victims in Japan. In 1968, these victims suffered an acute toxicosis from consuming rice oil contaminated with an industrial oil consisting of a mixture of PCBs, PCDFs and PCQs. The 581 males and 505 females sustained a total of 70 deaths (42 males vs. 45.81 expected and 28 females vs. 31.3 expected). These data are based upon Japanese national death rates over age 40 through October 31, 1983. In this population, for persons over 40 years of age overall cancer mortality was greater than expected in men but no different in women. In male Yusho victims 19 cancer deaths occurred vs. 11.50 expected and in female Yusho victims 7 cancer deaths occurred vs. 7.20 expected. However, by organ site, the risk of liver cancer was consistently found to be higher than expected in both men and women during the entire 16-year observation period. Even after a 9-16 year latent period, the risk of liver cancer in males was significantly higher (observed, 5; expected, 0.75; $p<.01$). This is a short latency period when compared with most known carcinogens. In females the results were 2 observed vs. 0.45 expected.

As a result of an in-depth review of this study and consideration of the overall PCDF toxicity relationships, it has been hypothesized by CAG that the PCDFs component of the PCBs could have been a factor in the statistically significant liver cancers seen in these victims. Particular isomers of the ingested PCDFs were found in liver tissue in nearly the same proportions as PCBs several years later (Kuratsune et al., 1975).

7.8. EPIDEMIOLOGY -- SYSTEMIC TOXIC EFFECTS

Two mass food poisonings, the first in Japan in 1968 and the second in Taiwan in 1979, were found to be caused by the ingestion of rice oil highly contaminated with PCB, PCDF and PCQ, but not with PCDD. The Japanese episode involved 1788 people at the end of 1982 and the Taiwan episode totaled 2062 patients at the beginning of 1983 (Masuda et al., 1985). It has been roughly estimated that the average intake during the whole intoxication period was 973, 3.8 and 586 mg in Taiwan (Chen et al., 1985a) and 633, 3.3 and 596 mg in Japanese patients (Hayabuchi et al., 1979) for PCB, PCDF and PCQ, respectively. The syndrome known as Yusho in Japan and Yu-Cheng in Taiwan, has been attributed mainly to PCDF, although the presence of PCB and PCQ should not be overlooked (Chen et al., 1981; Kashimoto et al., 1981). The animal data (Kashimoto et al., 1985; Kunite et al., 1985) indicate that PCB and PCQ components alone cannot cause Yusho symptoms.

Speculations on the levels of the three contaminant congeners detected in blood and tissues of Yusho and Yu-Cheng patients also support the hypothesis that PCDFs are the main agents responsible for the poisonings (Masuda et al., 1985; Miyata et al., 1977b, 1985; Chen et al., 1985a).

7.8.1. The Japanese Incident (Yusho disease).

Yusho disease has been described in several papers and comprehensive reviews (Kuratsune, 1972, 1975, 1980; Kimbrough, 1974; Hsu et al., 1985; Urabe and Asahi, 1985; Yoshimura and Hayabuchi, 1985). Therefore, only an overview of clinical effects will be given here.

The early symptoms presented by 89 males and 100 females in the Japanese Yusho incident are listed in Table 7-30. The available information allows only a qualitative description of the clinical effects; case control studies were aimed only at identifying the etiological role of rice oil. The associated estimates of the amount of oil consumed (ingested quantity of PCB and PCDF) and severity of clinical acute-subacute findings could be described in a few cases (see Table 7-14) (Kuratsune, 1972, 1975; Nagayama et al., 1976; Strik, 1979). Other available information will be briefly discussed.

7.8.1.2. VARIOUS CLINICAL AND BIOCHEMICAL FINDINGS -- Besides those reported for the acute and subacute phase after exposure (see Table 7-30), other abnormal nonsystematic findings are discussed in the following sections.

7.8.1.2.1. Liver -- Slight rises in serum transaminases and alkaline phosphatase, occasionally low bilirubin levels; marked proliferation of smooth endoplasmic reticulum in a liver biopsy sample from one exposed individual. Unspecified liver enlargement and disturbances were also reported.

7.8.1.2.2. Lipid Metabolism -- Serum triglycerides increased to four times the normal value, but plasma cholesterol and phospholipid levels were always normal. This hypertriglyceridemia persisted for several years. Follow-up of 24 patients showed lowered levels 5-7 years after the accident.

TABLE 7-30

Signs and Symptoms of Yusho Disease in Adult Japanese*

	Males (n=89) (%)	Females (n=100) (%)
SIGNS		
Blackening of nails	83.1	75.0
Black spots in all pores	64.0	56.0
Excessive sweating in palms	50.6	55.0
Acnelike skin eruption	87.6	82.0
Red spots on limbs	20.2	16.0
Change in skin color	75.3	72.0
Swelling of hands and feet	20.2	41.0
Hardening of backs of hands	24.7	29.0
Pigmentation of mucous membranes	56.2	47.0
Sebum (gum secretion in eyes)	88.8	83.0
Hyperemia of mucous membrane of eyes	70.8	71.0
Temporary failing of eyesight	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Fever	16.9	19.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0
SYMPTOMS		
Itching	42.7	52.0
Sense of weakness	58.4	52.0
Numbness of hands and feet	32.6	39.0
Hearing difficulty	18.0	19.0
Spasms of hands and feet	7.9	8.0
Headaches	30.3	39.0

*Source: Kuratsune, 1972

7.8.1.2.3. **Dermal Signs** -- The dermal lesions described in Table 7-30 were the most notable manifestations of Yusho. All skin symptoms diminished gradually but persisted with a tendency to cyst formation in severe cases. This was the initial criterion used to designate Yusho patients. The acneform eruptions decreased markedly after 3-4 years leaving a small number of cysts. Black comedones disappeared between 5 and 6 years: follicular dots disappeared within 4 years; pigmentation disappeared after 10 years, last from the nails. Ingrown nails are still apparent in 1985 (Urabe and Asahi, 1985).

Some women who consumed contaminated rice oil gave birth to "coco" (heavily black pigmented) babies (Kikuchi et al., 1969, 1977).

7.8.1.2.4. **Porphyrin Metabolism** -- Strik (1979) found no alteration of this factor in Yusho patients and no other manifestations of porphyria cutanea tarda such as light sensitivity or hyperkeratosis. Increased excretion of α-ketosteroid was an occasional finding and lower than normal immunoglobulin levels were reported in 38 patients 2 years after the accident.

7.8.1.2.5. **Ocular Signs** -- Although some signs subsided, 84% of 75 patients examined still showed abnormal changes of the meibomian glands 10 years after the accident; 64% of the patients complained of eye discharge.

7.8.1.2.6. **Neurological Signs** -- Reduced sensory nerve conduction velocity (9 of 23 cases) was observed more frequently than reduction of motor nerve conduction (2 cases only). No follow-up studies have been made. Dullness, headache, heavy-headedness, indefinite stomach ache, numbness, pains in the extremities, and swelling and pain of joints were still being reported in 1985 (Urabe and Asahi, 1985).

7.8.1.2.7. Respiratory -- Chronic bronchitis with mucus production was present in 40% of the cases. There was a very slow improvement in 79 patients that were followed for 5 years. Coughing and bronchitis-like symptoms were still being reported in 1985 (Urabe and Asahi, 1985). An investigation of 401 Yusho patients showed that one-half of the subjects were complaining of respiratory distress. Follow-up showed a gradual improvement over the 10 years following onset of the disease; however, from 10-15 years after onset little or no further improvement was observed in most cases (Nakanishi et al., 1985a,b) and some pulmonary function test impairment persisted long after the initial Yusho incident.

In conclusion, although the clinical profile of Yusho disease has been described in several papers, there is little precise and quantitative information on clinical manifestations, including the most severe.

7.8.2. The Taiwan Incident ("Yu-Cheng" Disease). On May, 1979, the first report of a Yusho-like disease in Taichung County, Taiwan was recorded with 1843 cases over a wide area up to November 1980 (Hsu et al., 1985). The initial diagnosis for 1670 victims was based on dermal phenomena set out in Table 7-23. By February 1983 there were 2062 recognized cases. In nine particularly afflicted townships, the morbidity rates were 0.095, 0.338, 0.392, 1.35, 0.248, 0.688, 0.056, 0.421 and 0.131%. The Kanechlor 400/Kanechlor 500 contamination in rice oil varied from 31-300 ppm, the period of exposure from 3-9 months and the estimated total intake per person from 0.77-1.84 g. The average latent period to the onset of visible signs was 3-4 months (range 1.5 to >6 months). In the first year, blood PCB in 613 patients ranged from 3-1156 ppb with 82.5% being 11-150 ppb; 27.6% had levels >100 ppb. Hyperpigmentation and growth retardation in newborn of pregnant mothers exposed to PCBs was noted along with enhanced mortality (weakness and lung problems). In adults, 24 deaths were reported mostly from hepatoma, liver cirrhosis or liver diseases with hepatomegaly.

PCB-exposed patients in the first year suffered from infections at the following sites: respiratory tract, skin, and immunosuppression (Lu and Wu, 1985). Ocular symptoms included increased discharge from eyes, swelling of eyelids, weakness of eyesight, soreness, easy irritation and easy fatigue of eyes (Lu and Wu, 1985). Other major complaints were hyperpigmentation (especially in nails) (Wong et al., 1985), headache, dizziness, general malaise, reduced appetite, soreness and weakness of limbs, swelling or pain of the joints and feet, ingrown nails (especially the big toe) and numbness of the limbs (Lu and Wu, 1985). Both sensory (in 43.6% of the patients) and motor (in 21.8% of the patients) nerve conduction velocity (NCV) were lower than control (Chen et al., 1985b). The major dermatological signs, as noted above, were acne-comedones, nail pigmentation and pruritis. Abnormal menstruation and hyperhydrosis of the palms and soles were noted in some females ~10%). Placental tissues obtained from women who had been exposed 3-4 years before conception, showed large increases in monoxygenase enzymes, particularly AHH (Wong et al., 1985). Males did not complain of impotence. Because of immunosuppression (humoral and cellular), secondary microbial infection often arose. Initially, total T, active T and helper T cells were decreased but not B or suppressor T cells. Three years later, only the helper T cells were still low giving an immuno-regulating index of 63% of unexposed controls. Studies 3 years later showed improvement of general conditions, of subjective symptoms and of cutaneous changes (Lu and Wu, 1985).

No association could be established between severity of the disease and PCB level in blood. However, the individual variations in the levels are so wide that no conclusion could be drawn from the reported results (Lu and Wu, 1985). Correlations between Yusho and Yu-Cheng symptomatology and PCDF levels in tissues have been discussed in previous sections.

7.8.3. The Binghamton, N.Y. Transformer Accident. An incident with explosion and fire in a tri- and tetrachlorinated benzene-containing electrical transformer took place in Binghamton, New York, on February 5, 1981. The consequent overheating led to the release of ~750 ℓ of fluid containing 65% PCBs and 35% tri- and tetrachlorinated benzenes. Pyrolytic conversion of these compounds led to the formation of several PCDFs and traces of PCDDs (Schecter et al., 1984). A total of ~500 persons were believed to be exposed, but data are available only on 50 patients who voluntarily sought medical surveillance. Among them, one case of chloracne, several transient skin rashes, three cases of skin cancer, three subjects with liver alterations with no other known cause were observed. Psychological symptoms (nervousness, irritability, fatigue, etc.) were recorded.

8. EFFECTS OF MAJOR CONCERN AND HEALTH HAZARD ASSESSMENT

8.1. EXISTING GUIDELINES AND STANDARDS

No guidelines, standards or recommendations are available for PCDFs.

8.2. SOURCES OF EXPOSURE

PCDFs enter the environment as unwanted trace impurities in commercial mixtures of polychlorinated biphenyls (PCBs), products derived from polychlorinated phenols (PCPs), chlorinated naphthalenes, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) formulations and through pyrolysis of PCBs, 2,4,5-T and polychlorinated diphenyl ethers. Incinerators (municipal, industrial and others), combusting organic materials and chlorinated compounds appear to a major source of PCDFs in the environment. The presence of PCDFs in the emissions from incinerators can be explained by the pyrolysis of chlorinated aromatic compounds. The chlorinated benzenes, PCBs and PCPs, which are present in high concentrations in emissions from waste incinerators, have also been suspected to act, either individually or in combination, as precursors or intermediates for PCDF formation. PCDFs emitted from incinerators include tetra-, penta-, hexa-, hepta- and octachlorodibenzofurans.

PCDFs are very widespread, appear to be highly stable in the environment like PCDDs, and can bioaccumulate in aquatic and terrestrial organisms. PCDFs have been detected in fish, turtles, seals, and fish-eating birds, in animal and human adipose tissue, and in animal and human milk. Exposure to the human population can occur by ingesting fish from contaminated water bodies. Human adipose tissue and breast milk from selected populations have been found to bioaccumulate PCDFs. Considerable concern has arisen over the public health impact of the emission of PCDFs from waste incinerators and presence of PCDFs in human adipose tissue and breast milk. Public health implications of nursing infants by mothers whose milk contains residues of PCDFs are many.

Accidental fires in electrical equipment, especially PCB-filled transformers and capacitors have been a source of exposure to PCDFs for a great number of people.

Wood preserved by chlorophenols is another source of PCDFs in the environment. High levels of PCDFs have been detected in dust from sawmills, sludge from wood dipping tanks, and other sources.

8.3. HIGH RISK POPULATIONS

Populations with high risk of exposure to PCDFs include the following: workers in the wood or tanning industry who produce or use chlorophenol preserved products; firefighters working on electric or transformer fires; workers at the incinerators; factory workers making or repairing transformers or capacitors, using casting waxes or heat exchange systems; office workers using carbonless copy papers containing PCBs; auto mechanics working on cars that use leaded gasolines; people who have a high intake of fish from PCDF contaminated water bodies; and infants who are nursed by mothers whose milk contains PCDF residues.

PCDFs are extremely toxic to animals and humans as demonstrated by short-term exposures. Long-term exposure and health experiences are notably lacking. Signs and symptoms of toxicity are very similar to those caused by 2,3,7,8-TCDD. Of the total 135 possible isomer congeners so far determined for PCDFs, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF seem to be most toxic. However, the relative toxicities of all the congeners have not yet been fully studied.

Information on adverse health effects in humans was gathered in 1968 and 1979 from observations in selected Japanese and Taiwanese populations, who accidentally consumed PCB-/PCDF-contaminated rice oil. Severe acute toxic effects were observed in the affected population. Retarded growth, abnormal lipid metabolism, liver dysfunction, acneform eruptions, skin pigmentation and cutaneomucosal lesions were some of the general symptoms observed in the affected individuals. Nine babies born of mothers from these affected populations had grayish/dark pigmentation of the skin ("coco" babies), gingival and nails, and the majority of these babies had an unusual discharge from the eye.

Liver and adipose tissue from affected populations examined several years after exposure showed retention of PCDFs at ppb levels; 2,3,4,7,8-PeCDF was found to be retained by the liver at higher concentrations than other isomers.

8.4. BASIS AND DETERMINATION OF RISK

There is no experimental evidence from human, animal or mutagenesis assays that pure PCDFs are carcinogenic or mutagenic in mammals. PCDFs do not give a mutagenic response in microbial assay systems. Furthermore, no long-term chronic human epidemiologic or animal bioassay toxicity data are available that can be used for determining human health risk from chronic exposure to pure PCDFs.

The only animal data available for hazard assessment result from short-term exposure. Birnbaum et al. (1986) and Birnbaum (1986) found 3 μg/kg/day to be the lowest observed effect level (LOEL) for hydronephrosis in the mouse fetus because of *in utero* exposure to 2,3,4,7,8-PeCDF during days 10-13 of the gestation period, while for cleft palate it was 5 μg/kg/day. The LOAEL for hydronephrosis for 2,3,7,8-TCDF, in a mouse teratogenicity study by Weber et al. (1984) was found to be 10 μg/kg/day.

Because of the short duration (4 days) for the in utero exposure to PCDFs resulting in hydronephrosis and cleft palate in the mouse fetus, the available teratogenicity data are not adequate to determine human health risk from chronic exposure. Consequently, the lowest-observed-adverse-effect level (LOAEL) is determined using the TCDD-equivalent extrapolation approach

8.4.1. Estimation of LOAELs. Structurally and toxicologically, PCDFs resemble their PCDD congeners. Their structural-activity relationships to AHH activity have been studied in a rat hepatoma cell line (Bandiera et al., 1984b; Mason et al., 1985). Like 2,3,7,8-TCDD, PCDFs need to bind to the cytosolic Ah receptor site for manifestation of their AHH inducing activity. The chlorine atoms have to occupy at least three of the four lateral positions (2, 3, 7 and 8) and at least one vicinal carbon position must be unsubstituted. A linear relationship for a series of PCDFs, a PCDF mixture typical of exposure to Yusho patients and 2,3,7,8-TCDD was obtained when -log EC_{50} values for AHH induction responses were plotted against -log ED_{50} values for body weight loss or thymic atrophy (Mason et al., 1985). In all cases 2,3,7,8-TCDD was found to be the most active substitute for AHH induction in vitro and for in vivo short-term toxic effects.

Taking into consideration the AHH activity and other acute toxic effects, it has been estimated that 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF are 3- and 20-fold, respectively, less toxic than 2,3,7,8-TCDD (Bandiera et al., 1984b; Mason et al., 1985) (see Table 7-29 and Figure 7-1).

Reevaluation of the Murray et al. (1979) primary data from a 3-generation Sprague-Dawley rat reproductive effect study with 2,3,7,8-TCDD by Nisbet and Paxton (1982) and subsequently by U.S. EPA (1985) revealed that the LOAEL for 2,3,7,8-TCDD was 0.001 µg/kg/day.

Toth et al. (1978, 1979) exposed male Swiss mice to 0.007 µg/kg/week of 2,3,7,8-TCDD for 1 year, which resulted in amyloidosis of the kidney, spleen and liver, and dermatitis at the time of death. The duration of this study was 649 days. In this study 0.001 µg/µg/day was also found to be the LOAEL.

The LOAELs for 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF are estimted to be 0.003 µg/kg/day and 0.02 µg/kg/day, respectively, from the above animal long-term chronic studies using the TCDD equivalent approach.

For comparative purposes, an attempt will now be made (in consultation with Dr. J.J. Ryan) to determine the LOAEL for 2,3,4,7,8-PeCDF in the Yusho poisoning incident.

The minimum quantity of rice oil that led to an observed effect (grade 3 Yusho response) in a 42-year-old person after a latent period of 30 days was 121 mℓ. The mean latent period in the Yusho episode was estimated to be 71 days (range: 20-190 days) (Hayabuchi et al., 1979). The average amount of total PCDFs in the rice oil has been estimated (Ryan, 1986) from several sources (Buser et al., 1978; Miyata et al., 1978 and Maguda, 1982) (see Chapter 4) to be 3.85 µg/g (ppm).

Using the peak heights on the chromatogram in the Buser et al. (1978) study, from peak 68 it has been estimated that 2,3,4,7,8-PeCDF was ~8.4% of the total PCDFs of the Yusho oil (Ryan, 1986). The content of 2,3,7,8-TCDF in the oil is assumed to be negligible since both Masuda et al. (1982) and Chen and Hites (1983) have found that the peak eluting near 2,3,7,8-TCDF is in fact 2,3,4,8-TCDF. Moreover, the contribution of 1,2,3,4,7,8- and 1,2,3,6,7,8-HxCDF is not considered to be significant since: a) HxCDFs were present in lower concentrations than PeCDFs in the oil, b) HxCDFs accumulated less in tissues of Yusho patients than PeCDFs (Kuroki and Masuda, 1978), and c) the HxCDFss are less toxic than PeCDFs in animals.

Using the estimations on the total oil consumed, the average quantity of total PCDFs in the oil, and the percentage of 2,3,4,7,8-PeCDF in the total PCDFs in the oil, the LOAEL in a 70 kg man for 2,3,4,7,8-PeCDF is determined to be:

$$\frac{121\ \text{m}\ell \times 3.85\ \mu g \times 0.084}{71\ \text{days} \times 70\ \text{kg}}$$

$$= 0.007\ \mu g/kg/day$$

For 2,3,4,7,8-PeCDF, this estimated LOAEL of 0.007 μg/kg/day from the Yusho incident is supportive of the LOAEL of 0.003 μg/kg/day derived from animal studies using the TCDD equivalent approach.

8.4.2. Derivation of Reference Dose (RfD). In the absence of long-term chronic animal bioassay data on 2,3,4,7,8-PeCDF it seems reasonable to determine a Reference Dose (RfD) based on the LOAEL estimated following the TCDD equivalent approach. A composite uncertainty factor of 1000 is used to estimate the RfD. This uncertainty factor represents 10 because adverse effects observed in animals are extrapolated to humans, and another 10 is used to account for the expected interhuman variability in the toxicity response to this chemical, an additional uncertainty factor of 10 is used because the RfD is based on a LOAEL and not a NOAEL; thus, this latter factor adjusts the LOAEL into the range of the expected NOAEL. An uncertainty factor of 10 to account for the short duration of the study is not used because the critical toxic effect, teratogenicity, is encompassed in the duration of the study. Consequently, an uncertainty factor of 100x10=1000 is used.

8.4.2.1. THE RfD FOR 2,3,4,7,8-PeCDF -- The RfD for 2,3,4,7,8-PeCDF is estimated to be as follows:

$$\frac{0.003\ \mu g/kg/day}{1000} = 3 \times 10^{-6}\ \mu g/kg/day$$

The following is a series of calculations that estimate the criteria for 2,3,4,7,8-PeCDF in various media.

1. For a 70 kg man inhaling 2,3,4,7,8-PeCDF in 20 m³ of air/day the criteria would be:

$$= \frac{3 \times 10^{-6}\ \mu g/kg/day \times 70\ kg}{20\ m^3/day}$$

$$= 1.1 \times 10^{-5}\ \mu g/m^3$$

2. For a 50 kg woman inhaling 2,3,4,7,8-PeCDF in 20 m³ of air/day

$$= \frac{3 \times 10^{-6}\ \mu g/kg/day \times 50\ kg}{20\ m^3/day}$$

$$= 7.5 \times 10^{-6}\ \mu g/m^3$$

[The above calculations assume that exposure occurs only by inhalation.]

3. Since 2,3,4,7,8-PeCDF residues have been detected in mother's milk samples from isolated populations, a virtually safe level for ingestion of 2,3,4,7,8-PCDF in mothers' milk is also estimated. In this calculation, the total body weight of an infant is considered to be 10 kg (ICRP, 1975). The total quantity of fat in 100 mℓ of mother's milk is 4.5 g and the average daily intake of nursing mother's milk by the baby is considered to be 850 mℓ (ICRP, 1975). Therefore, 850 mℓ of mother's milk will contain 38.25 g of fat.

   Therefore, for virtual safety for an infant, the concentration of 2,3,4,7,8-PeCDF in fat from the nursing mother's milk should not exceed the following:

$$\frac{3 \times 10^{-6}\ \mu g/kg/day \times 10\ kg}{38.25\ g/day}$$

$$= 7.8 \times 10^{-7}\ \mu g\ 2,3,4,7,8\text{-PeCDF/g of fat.}$$

4. Since fish has been found to bioaccumulate PCDFs easily, a criterion for a 70 kg man from ingestion of contaminated fish is also determined. The average daily consumption of fish in the United States is considered to be 6.5 g (Federal Register, 1980).

Therefore, the criterion is as follows:

$$\frac{3 \times 10^{-6}\ \text{mg/kg/day} \times 70\ \text{kg}}{6.5\ \text{g/day}}$$

$$= 3.2 \times 10^{-5}\ \text{mg 2,3,4,7,8-PeCDF/g of fish.}$$

This calculation is based on fish as the sole source of 2,3,4,7,8-PeCDF.

8.4.2.2. THE RfD FOR 2,3,7,8-TCDF -- Following TCDD equivalent approach from animal data, the LOAEL for 2,3,7,8-TCDF has been estimated (Section 8.4.1.) to be 0.02 µg/kg/day. Based on this LOAEL, in the absence of any chronic animal bioassay data on 2,3,7,8-TCDF it seems reasonable to determine a RfD for this compound.

A composite uncertainty factor of 1000 is used to estimate the RfD. This uncertainty factor represents 10 because adverse effects observed in animals are extrapolated to humans, and 10 to account for the expected interhuman variability in the toxicity response to this chemical, an additional uncertainty factor of 10 because the RfD is based on a LOAEL and not a NOAEL; thus, this latter factor adjusts the LOAEL into the range of expected NOAEL. An uncertainty factor of 10 to account for the short duration of the study is not used because the critical toxic effect, teratogenicity, is encompassed in the duration of the study. Consequently, an uncertainty factor of 100x10=1000 is used.

The RfD is estimated to be:

$$\frac{0.02\ \mu\text{g/kg/day}}{1000} = 2 \times 10^{-5}\ \mu\text{g/kg/day}$$

The following is a series of calculations that estimate criteria for 2,3,7,8-TCDF in various media.

1. For a 70 kg man inhaling 2,3,7,8-TCDF in 20 m^3 of air/day the criteria would be

$$= \frac{2 \times 10^{-5}\ \mu g/kg/day \times 70\ kg}{20\ m^3/day}$$

$$= 1 \times 10^{-4}\ mg/m^3$$

2. For a 50 kg woman inhaling 2,3,7,8-TCDF in 20 m^3 of air/day

$$= \frac{2 \times 10^{-5}\ mg/kg/day \times 50\ kg}{20\ m^3/day}$$

$$= 1 \times 10^{-4}\ \mu g/m^3 \text{ air.}$$

[The previous calculations assume that exposure occurs only by inhalation.]

3. Since 2,3,7,8-TCDF residues have been detected in mother's milk samples from specific populations, a virtually safe level for ingestion of 2,3,7,8-TCDF in mothers' milk is also estimated. In this calculation, the total body weight of an infant is considered to be 10 kg (ICRP, 1975). The total quantity of fat in 100 mℓ of mother's milk is 4.5 g and the average daily intake of nursing mother's milk by the baby is considered to be 850 mℓ (ICRP, 1975). Therefore, 850 mℓ of mother's milk will contain 38.25 g of fat.

Therefore, for virtual safety for an infant, the concentration of 2,3,7,8-TCDF in fat of the nursing mother's milk should not exceed

$$\frac{2 \times 10^{-5}\ \mu g/kg/day \times 10\ kg}{38.25\ g/day} = 5.2 \times 10^{-6}\ \mu g\ \text{2,3,7,8-TCDF/g of fat.}$$

4. Since fish has been found to bioconcentrate PCDFs easily, a criterion for a 70 kg man from ingestion of contaminated fish is also determined. The average daily consumption of fish in the United States is considered to be 6.5 g (Federal Register, 1980).

Therefore, the criterion is as follows:

$$\frac{2 \times 10^{-5}\ \mu g/kg/day \times 70\ kg}{6.5\ g/day} = 2 \times 10^{-4}\ \mu g\ \text{2,3,7,8-TCDF/g of fish.}$$

These estimations provide general guidance only. To determine a more accurate criteria it will be necessary to do a comprehensive exposure assessment taking into consideration the exposure through various compartments of the environment and valid chronic bioassay data on 2,3,4,7,8-PeCDF and 2,3,7,8-TCDF.

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